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NOTES ON AFRICAN *HAEMAPHYSALIS* TICKS. I. THE MEDITERRANEAN-LITTORAL HEDGEHOG PARASITE *H. ERINACEI* PAVESI, 1884 (IXODOIDEA, IXODIDAE)

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Haemaphysalis erinacei Pavesi, 1884, a hedgehog parasite of North Africa and possibly also of the Near East, has been known from fewer than a dozen specimens from widely scattered localities. Large series of well preserved material are especially necessary for basic morphological study of this species in view of the recent proposal to separate North African from Palestinian-Russian populations on the basis of the numbers and length of coxal hairs (Feldman-Muhsam 1953), a unique species criterion for ixodid ticks.

Although this tick is common only in a narrow range of Egyptian localities, we have been able to obtain good series for distributional, biological, and morphological studies, which are reported herein. The immature stages are described and illustrated for the first time.

HAEMAPHYSALIS ERINACEI Pavesi, 1884

(Figures 1 to 35)

Haemaphysalis erinacei Pavesi, 1884, pp. 484, description of single male (and "females" which Feldman-Muhsam (1953, p. 372) has found to be nymphal *Hyalomma*), from Algerian hedgehog, *Erinaceus algirus* subsp., Gebel Resas, Tunisia. Type (and "female types") in Museum of Genoa, Italy. Feldman-Muhsam (1953, pp. 372–378) reviewed taxonomy, illustrated male (figs. 1 and 2) and female (figure 3), and discussed morphology (see DISCUSSION below). Hoogstraal (1954, p. 308) incorrect report of this species from Anglo-Egyptian Sudan, based on old error in identification.

Haemaphysalis leachii (Audouin, 1827). Neumann (1897, p. 349) original identification of specimens later used as type material for *H. numidiana*. Colas-Belcour and Rageau (1951, p. 365) specimens from *E. algirus* from Tunis, do not recognize *H. "numidiana"* as a valid species; some of these specimens presented to Hoogstraal collection.

Haemaphysalis numidiana Neumann, 1905, p. 230, description of male and female (actually first female description of *H. erinacei*), from 3 males and 1 female from hedgehog, Tebessa, Algeria; material at Toulouse Veterinary School [one male cotype in British Museum (Natural History, Nuttall lot 2890)]. Warburton (1933, p. 567) and Cooley (1934, p. 31) report species from South Africa; this is considered questionable (see DISCUSSION below). Feldman-Muhsam (1953, pp. 372–378) synonymized *H. numidiana* under *H. erinacei* Pavesi, 1884. For other subspecies and extra-African reports and data, see Feldman-Muhsam (1953).

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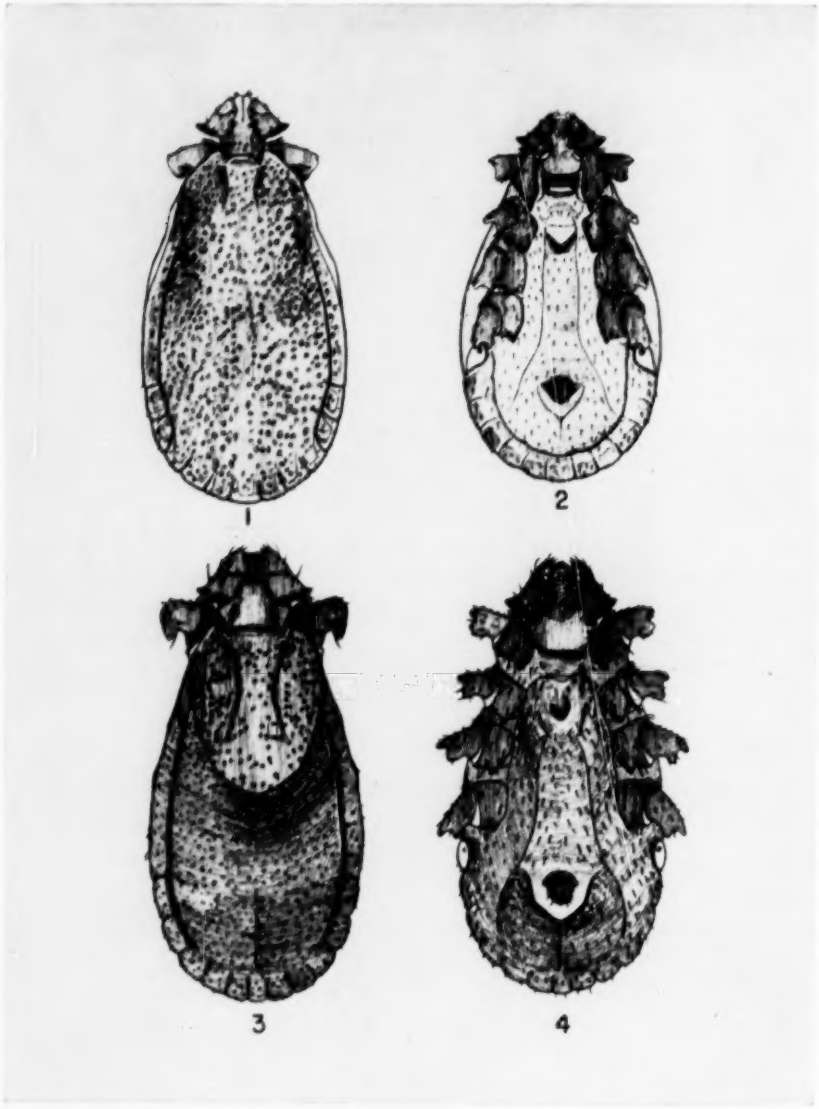
The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Haemaphysalis crinacei Feldman-Muhsam, 1951 (as new species), pp. 105–106, description of single male from hedgehog, Jerusalem; figure 10. Feldman-Muhsam, 1953, pp. 372–378, synonymy under *H. crinacei* Pavesi, 1884.

MATERIAL EXAMINED. Total: Many eggs, 16 wild-caught larvae, ±625 reared larvae,



FIGS. 1–4. *Haemaphysalis crinacei* Pavesi, 1884. Egyptian specimens (Western Desert). 1, male, dorsal view; 2, male, ventral view; 3, female, dorsal view; 4, female, ventral view.

±162 wild-caught nymphs, 1 reared nymph, 61 wild-caught males, 22 reared males, 43 wild-caught females, 46 reared females.

ALGERIA: Timgad, 17 nymphs from dipodil gerbil, Gerbillus (*Dipodillus*) *campestris*

subsp. (2 hosts), 12 and 17 April 1920, K. Jordan gift, Nuttall lots 3537 and 3739 (which had been identified as *H. leachii*) in British Museum (Natural History).

TUNISIA: Tunis, 2 males from Algerian hedgehog, *Erinaceus a. algirus* Duvernoy and Lereboullet, 1848, collected April, 1929 (kindly presented by Dr. J. Colas-Belcour).

PALESTINE: Jerusalem, 1 male from European hedgehog, *Erinaceus europaeus transcaucasicus* Satunin, 1905 (syn. *E. roumanicus sacer* Thomas, 1918), 3 March 1922, P. A. Buxton *legit*, Nuttall lot 3482a (which had been identified as *H. leachii*) in British Museum (Natural History).

EGYPT (WESTERN DESERT): Unless otherwise specified, all specimens are from single hosts, the Egyptian long-eared hedgehog, *Hemiechinus auritus aegyptius* (Fischer, 1829), collected between 1951 and 1954 by H. Hoogstraal with the assistance of A. A. Salah and M. N. Kaiser.

Alexandria: 18 nymphs, 19 March; 1 female, 25 July. *Amirya:* 1 male, 24 September. *Burg el Arab:* 1 male, 4 females, 30 January (2 hosts); ± 119 nymphs, 28 males, 15 females, various dates in April (six hosts); 16 larvae, 2 males, 1 female, 24 May (2 hosts); 2 nymphs from jird, *Meriones s. shawi* (Duvernoy, 1842), 5 April; 1 male from fox den, 30 January. *El Alamein:* 12 males, 12 females, April. *El Daba:* 1 nymph, 31 April; 1 female, 1 May; 1 nymph, 1 male in old hedgehog burrow on desert, 31 April. *Ras el Hekma:* 4 males, 3 females, 20 April. *Mersa Matruh:* 4 females, 1 May; 1 female, 11 September; 9 nymphs from one hedgehog burrow (with *Ornithodoros arenicolous*) examined in April and May of successive years. *Sidi Barrani:* 2 nymphs, 8 males, 1 female, various dates in April (4 hosts). *Salum* (Libyan Plateau above village): 1 nymph from dipodil gerbil, *Gerbillus* (*Dipodillus*) *campestris* subsp., 28 October; 1 nymph from dormouse, *Eliomys quercinus* subsp., 24 October; from desert 19 miles east of village: 1 nymph from dipodil, *Gerbillus* (*Dipodillus*) sp. 14 January.

REARED MATERIALS: Original specimens collected on Egyptian long-eared hedgehogs, Burg el Arab, April, 1953 (totals listed above; further data in DISCUSSION below).

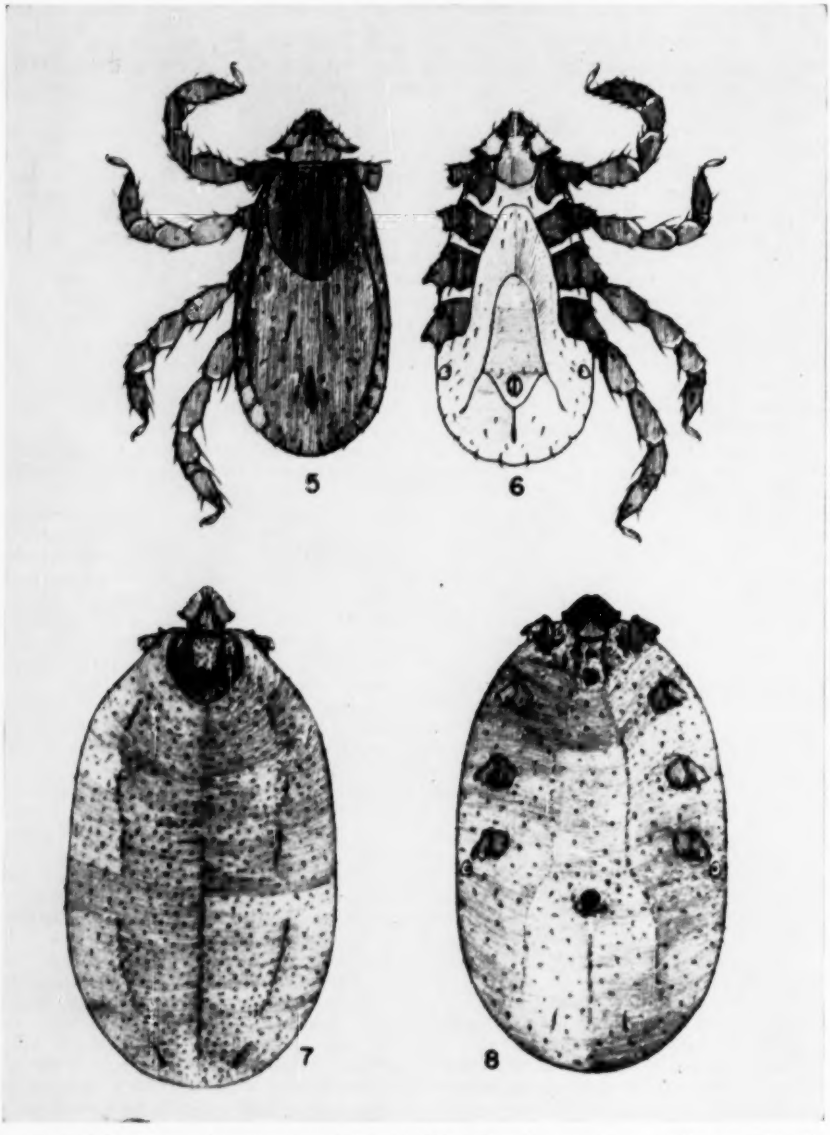
REDESCRIPTION. MALE (reared from wild nymphs and freshly preserved): *Length* overall from 2.4 mm. to 3.6 mm., width from 1.4 mm. to 2.2 mm. *Body* elongate oval, widest just posterior of midlength, gradually converging from widest level to scapulae, posterior margin broadly rounded. *Color* pale yellow to reddish brown.

Capitulum: Basis capituli rectangular, with lateral margins parallel or only very slightly diverging anteriorly, measuring about 0.21 mm. long including cornua and 0.34 mm. wide; surface with a few shallow punctations; posterior margin between cornua straight or imperceptibly concave; cornua from one-third to one-half as long as basis, forming about an equilateral triangle, bluntly or sharply pointed. *Palpi* basally salient, measuring about 0.26 mm. at greatest length and 0.66 mm. at point of greatest width of both palpi; basal margin dorsally and ventrally forming a somewhat tilted straight line (without angles or spurs); basal and lateral margins meeting at an acute angle which may be slightly upturned; lateral margins straight, rarely slightly concave, and gradually converging to comparatively narrow apex; segment 3 from two-fifths to one-third as long as segment 2, ventrally with a narrow, elongate, sharply or bluntly tipped retrograde spur extending over the anterior half of segment 2. *Hypostome* with 4/4 dentition, in files of seven to nine (mostly eight) denticles most of which are sharply pointed and strongly recurved; apex bluntly rounded and with distinct corona.

Scutum: Lateral grooves deep and narrow, posteriorly enclosing anterior two pairs of festoons, anteriorly extending at least to anterior third of scutal length. *Cervical grooves* moderately deep, extending to about anterior level of lateral grooves, converging and deeper anteriorly, diverging and more shallow posteriorly. *Festoons* clearly marked; length and width ratio variable. *Scutal suface* slightly convex; punctations moderately numerous, mostly non-contiguous, distributed over entire surface including lateral areas and festoons, shallow and non-discrete; size either uniformly moderately large throughout or moderately large anteriorly to small posteriorly; foveae usually distinct.

Legs: Coxae elongate, each with a small but distinct posterior spur, that of I at the inner margin and almost reaching II; those of II and III situated just laterad of inner margin and reaching the anterior margins of III and IV respectively; that of IV of about equal length, slightly narrower, at or just laterad of inner margin. *Coxal hairs* as follows: four or five of intermediate length along outer margin anteriorly, three intermediate to long length along posterior margin between outer edge and inner spur (I with six or seven hairs in this situation), three to five short to long length along inner margin (these short on I, intermediate on II and III, and long on IV); one to three short to intermediate length centrally near outer margin; total number of hairs on each coxa from 12 to 17 of which those along the anterior outer margin and along the basal margin are generally the longest groups. *Trochanters* unarmed ventrally; I dorsally with a moderate shield. *Tarsi* stout, II to IV with a very small basal hook apically (hook minute to obsolete in some specimens); I with length and width about equal,

fairly abruptly tapered almost from base to apex; II to IV with dorsal margins paralleling ventral margins or slightly humped, apicaly truncate, fairly abruptly tapering from about midlength to apex; I with three short lateral pairs of hairs, four slightly longer ventral pairs, and three dorsal pairs; other tarsi with five short lateral pairs, six longer basal pairs, and three dorsal pairs of varying length. *Pads* (*pulvilli*) large, reaching almost to apical sixth of claws. *Claw* I longer than tarsus I; other claws about about five-sevenths as long as tarsi bearing them.

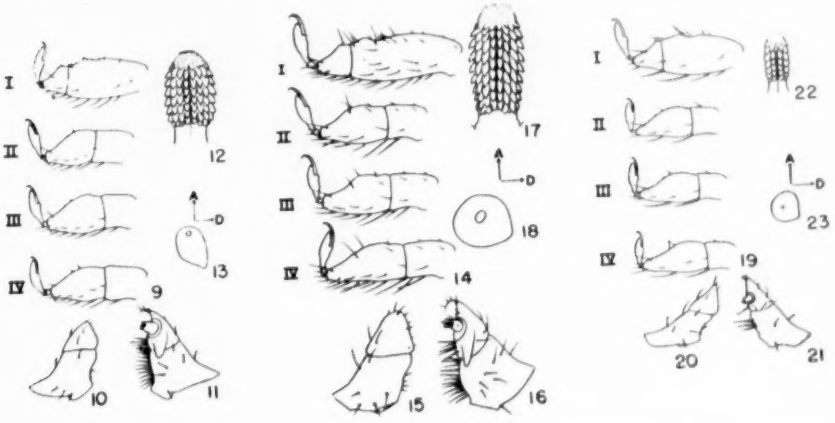


FIGS. 5–8. *Haemaphysalis erinacei* Pavesi, 1884, Egyptian specimens (Western Desert). *5*, nymph, dorsal view; *6*, nymph, ventral view; *7*, female, greatly engorged, dorsal view, showing lateral stretching and rugosity of scutum with extreme engorgement; *8*, female greatly engorged, ventral view.

Ventral surface: Integument with short, regularly spaced hairs as illustrated. *Genital aperture* large, situated level with anterior half of coxae II, apron strongly and irregularly serrated. *Genital* and *anal grooves* distinct, as illustrated, anal grooves usually not clearly extended anteriorly to genital grooves or posteriorly to festoon. *Spiracular plate* widely oval with a small dorsal extension; directed dorso-caudally.

FEMALE. In primary characters, the female closely approximates the male. *Length* overall, unengorged 2.97 mm. to 3.63 mm.; *width* 1.61 mm. to 1.99 mm.; length engorged up to 8.25 mm.; width 4.95 mm. Body shape unengorged similar to that of male. Body when engorged with parallel lateral margins; scutum on anterior fifth of body, stretched laterally, and surface distinctly rugose; coxae widely separated.

Capitulum: Basis capituli with lateral margins almost parallel or slightly diverging, measuring 0.264 mm. long including cornua and 0.429 mm. wide; porose areas deep, tilted, wide ovals, distant from each other; posterior margin straight or slightly concave; cornua moderate, bluntly triangular. *Palpi* more elongate but otherwise similar to those of male; measuring about 0.363 mm. at greatest length and 0.770 mm. at point of greatest width of both palpi. *Hypostome* with 4/4 dentition, more elongate than that of male, with apex more bluntly rounded and with files of eight to ten (mostly nine) denticles.



FIGS. 9–23. *Haemaphysalis erinacei* Pavesi, 1884, Egyptian specimens (Western Desert). 9, male, tarsi I to IV, lateral view; 10, male, palpus, dorsal view; 11, male, palpus, ventral view; 12, male, hypostome, ventral view; 13, male, spiracular plate; 14, female, tarsi I to IV, lateral view; 15, female, palpus, dorsal view; 16, female, palpus, ventral view; 17, female, hypostome, ventral view; 18, female, spiracular plate; 19, nymph, tarsi I to IV, lateral view; 20, nymph, palpus, dorsal view; 21, nymph, palpus, ventral view; 22, nymph, hypostome, ventral view; 23, nymph, spiracular plate.

Scutum: Outline (unengorged) with lateral margins widely diverging from rounded scapulae to level of anterior fourth of scutal length, thence gradually converging to rather narrowly and unevenly rounded posterior margin; measuring 1.287 mm. long and 0.924 mm. wide at anterior fourth. With engorgement the scutum stretches laterally and length and width are about equal; the surface becoming markedly rugose (see figure 7). *Cervical grooves* moderately deep, converging almost to scutal midlength, thence diverging to posterior fourth of scutum. *Punctations* medium-size to large, non-discrete, some with a short hair; fairly numerous in lateral fields, few centrally and posteriorly.

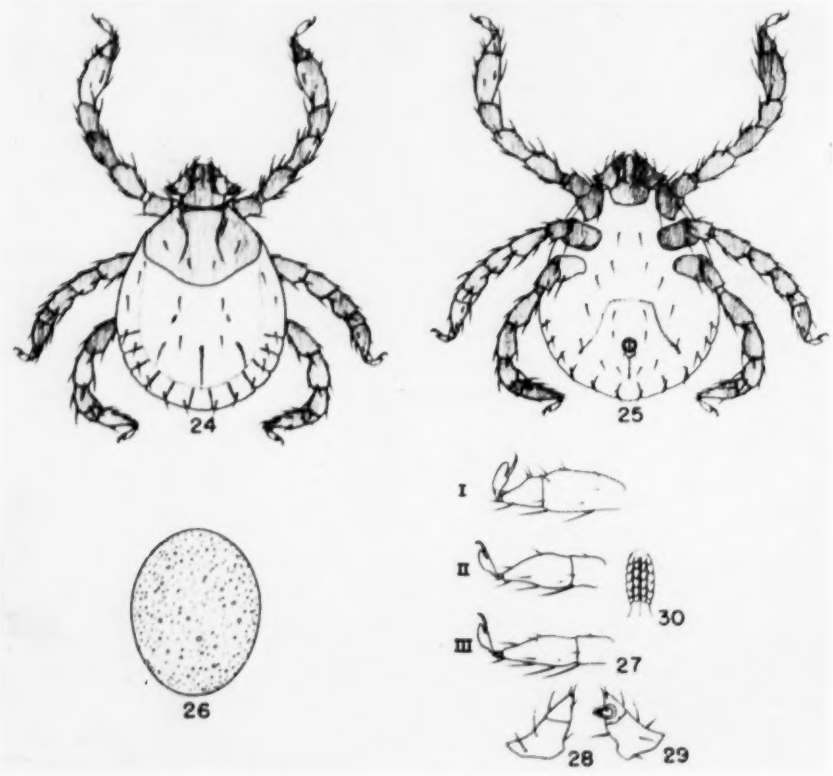
Legs: Coxae more rectangular and distant from each other, but otherwise similar to those of male. Coxal hairs longer than those of male, equalling those of male in position and numbers except that on I they are more numerous (eight or nine long) along the outer margin anteriorly and along the basal margin (eight or nine medium to long). *Tarsi* more elongate, with longer hairs, ventral apical hooks minute to obsolete; otherwise similar to those of male. *Pads* (*pulvilli*) not quite as long in relation to length of claws as those of male, but difference amounting to only a very small fraction. *Claws* with length in comparison with tarsal length as in male.

Ventral surface: Integument with more and longer hairs than on male. *Genital aperture* situated between coxae II (unengorged), or just posterior of coxae I (engorged). *Genital*

and *anal grooves* distinct, as illustrated. *Spiracular plate* subcircular with a small, blunt dorsal extension.

NYMPH: The nymph is readily identifiable with adults. Length and width of large specimens may equal that of small or moderate size males. The body shape, unengorged, like that of adults; engorged, like that of females.

Capitulum: Basis capituli very short and wide (almost three times as wide as long including cornua), lateral margins slightly diverging anteriorly, posterior margin slightly concave; cornua very small, broadly triangular points. *Palpi* extremely salient basally, 2.5 times as wide as long; outline otherwise as in adults; segments 2 and 3 of about equal length; segment 3 ventrally with a very small retrograde spur barely reaching the basal margin of segment 3. *Hypostome* with 2/2 dentition of six or seven denticles in each file; corona present; apex bluntly rounded.



FIGS. 24–30. *Haemaphysalis erinacei* Pavesi, 1884, Egyptian specimens (Western Desert). 24, larva, dorsal view; 25, larva, ventral view; 26, egg, drawn to same scale as larva; 27, larva, tarsi I to IV, lateral view; 28, larva, palpus, ventral view; 29, larva, palpus, ventral view; 30, larva, hypostome, ventral view.

Scutum. Outline as in female but more gradually rounded and more pointed posteriorly. *Cervical grooves* as in female but extending to posterior margin of scutum. Punctations almost inapparent but indicated by 10 to 20 short hairs.

Legs: Coxae with outlines similar to those of females, spurs present but reduced as illustrated. Coxal hairs as follows: two or three intermediate to long along outer margin anteriorly; one intermediate to long basally, three to five short to intermediate in various positions elsewhere; total number of hairs on each coxa from six to nine, mostly intermediate to long. *Tarsi* intermediate between those of male and female; each with at least three pairs of ventral, lateral, and dorsal hairs. *Pads* and *claws* intermediate between those of males and females.

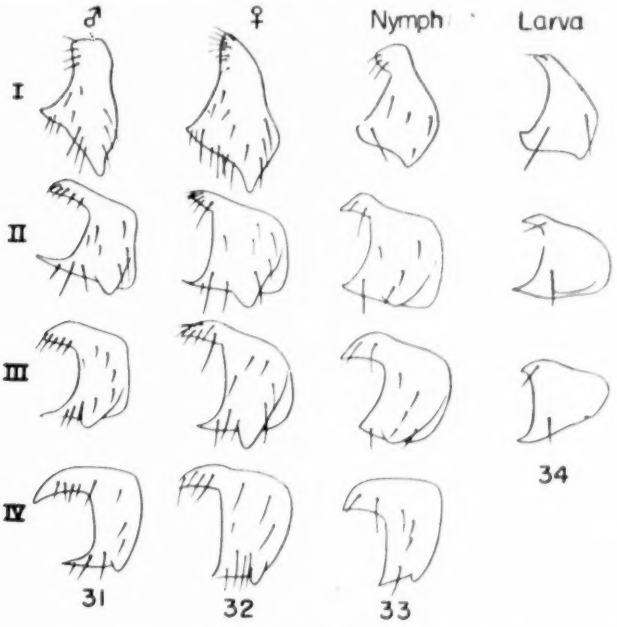
Ventral surface as illustrated.

LARVA (Unengorged): Length from apex of palpi to posterior body margin about 0.75 mm.; width about 0.51 mm. Body outline subcircular.

Capitulum: Basis capituli rectangular, about three times as wide as long; cornua obsolete or almost so. *Palpi* with outline and proportions similar to those of nymph except that segment 3 is shorter in comparison with segment 2, and the ventral spur of segment 3 is much reduced. *Hypostome* with 2/2 dentition in files of six denticles; corona present, apex gradually rounded.

Scutum: Outline wide, two-thirds as long as wide; widely diverging to midlength; short, rounded lateral margins; posterior margin bilaterally concave. *Cervical grooves* converging to midlength of scutum, thence diverging and extending almost to posterior margin. Punctations indicated only by two hairs in each lateral field and in median field posteriorly.

Dorsal integument with lateral groove continuous; festoons clearly indicated and each except middle one bearing a single hair; two other pairs of hairs in lateral areas and on median



FIGS. 31-34. *Haemaphysalis erinacei* Pavesi, 1884, Egyptian specimens (Western Desert). 31, male, coxae I to IV; 32, female, coxae I to IV; 33, nymph, coxae I to IV; 34, larva, coxae I to IV.

dorsum. *Ventral integument* with festoons somewhat less clearly marked, each except middle one with a single hair; with a longitudinal row of five hairs between median line and coxae on each side; three pairs of hairs bordering anus; one hair on each anal valve; and one pair of hairs midway between anus and festoons; postanal median groove faintly indicated.

Legs: Coxae each with two long hairs, three on I; I with a small, blunt spurlike point at juncture of inner and posterior margins; II with raised ridge posteriorly; III with all but obsolete spurlike point in same position as that of I. *Tarsi* with two or three pairs of ventral hairs, two pairs of lateral hairs, and two or three pairs of dorsal hairs; I five-sixths as wide as long; II and III about twice as long as wide; all gradually tapered from about midlength. *Claw* I slightly longer than tarsus I, II and III about two-thirds as long as their respective tarsi. Pads moderately large.

EGG. Shape widely ovoid, one end very slightly narrower than the other, about 0.50 mm. long, 0.37 mm. wide. Color yellow.

DISCUSSION AND CONCLUSIONS

MORPHOLOGY: Feldman-Muhsam (1953), who recently compared the type specimens of *Haemaphysalis erinacei* Pavesi, 1884, with those of *H. numidiana* Neumann, 1905, found that they represented a single species. The specimens had been collected in Tunisia and Algeria, respectively. With these, she compared Palestinian material, which she (1951) had named *H. erinacei* (nec *erinacei* Pavesi, 1884), and concluded, on the basis of differences in length and number of coxal hairs, that these represented *H. taurica* Pospelova-Shtrom. The five North African type specimens and two of our Egyptian wild-caught specimens which she had received, were considered to have fewer and shorter coxal hairs (*H. erinacei*) than the Palestinian-Russian species (*H. taurica*).

From study of our rather large number of specimens collected on Egyptian hedgehogs or reared from nymphs and preserved while in excellent condition, it appears that this criterion, which is quite a plausible one, must be very carefully handled. Our specimens removed from hedgehogs show a wide range of coxal hair length and numbers, based obviously on wear and tear while the tick was alive. Our material reared from nymphs collected from the same hedgehog hosts, shows constancy of coxal hair length and numbers (within the limits mentioned in our description of each sex and stage). They equal what Feldman-Muhsam (1953) considered to be the long and numerous-haired Palestinian-Russian species, *H. taurica* Pospelova-Shtrom.

Our two Egyptian specimens, which Feldman-Muhsam (1953) had identified as *H. erinacei* (few, short hairs), were collected before we commenced rearing this species in the laboratory. They are from a lot others of which Feldman-Muhsam would obviously call *taurica*. All of our reared Egyptian material equals her specifications for *taurica*.

Feldman-Muhsam (1953) illustrated for the male type of *H. erinacei* (from Tunisia), five to seven hairs on each coxa (five on I, five on II, seven on III, six on IV) of which most are "short" (by comparison with Egyptian and Palestinian specimens) and one or two are intermediate length. For the female of *H. erinacei* (type of *H. numidiana* from Algeria) she illustrated 12 short to moderate hairs on I and six to eight mostly short to moderate on the other coxae.

The two males from Tunisia in my collections, both obviously rubbed and with some broken hairs, have on the four coxae in a homologous position the following hairs:

I six to nine, intermediate to long.
II four to nine, short to long.
III five to nine, short to long.
IV three to seven, short to long.

Our two obviously worn Tunisian males, therefore, have longer and more numerous coxal hairs than specified by Feldman-Muhsam for the (Tunisian) type of *H. erinacei*, and approach Egyptian non-worn specimens in both numbers and length of coxal hairs. Egyptian worn specimens in some instances equal the two Tunisian ones in hair length and numbers.

The single British Museum (Natural History) specimen from Palestine is indistinguishable from Egyptian specimens in any features.

It appears, therefore, that there may be some question as to actual variation in length and numbers of coxal hairs in populations of haemaphysalid ticks parasitizing hedgehogs from Algeria to Palestine. Larger series of carefully preserved, freshly molted specimens from throughout this area are necessary for study before final conclusions can be drawn.

TAXONOMIC CONCLUSIONS: As a result of the above studies, we have at the present time no recourse but to consider the hedgehog parasites of North Africa

FIG. 35. Map of Egypt, to show distribution of hedgehogs and collecting localities of *Haemaphysalis erinacei*.

and possibly of Palestine as a single species, *H. erinacei* Pavesi, 1884 (syn. *H. numidiana* Neumann, 1905). The question of the actual relationships of *H. erinacei* to *H. taurica* Pospelova-Shtrom, of Russia remains unsettled.

HOSTS: In Egypt, the only known host of the adult stage of *H. erinacei* is the Egyptian long-eared hedgehog, *Hemiechinus auritus aegyptius* (Fischer, 1829). About one in seven of these hedgehogs that we have examined from the Mediter-

ranean littoral between Alexandria and the Libyan frontier has been found infested. Hundreds of long-eared hedgehogs from other parts of the range of this animal in Egypt (see Figure 35 and BIOLOGY below) have been uninfested by *H. erinacei*.

None of the various kinds of *Paraechinus* hedgehogs known from Egypt have been found infested. Although only 25 specimens of *Paraechinus* hedgehogs have ever been collected throughout Egypt, all of them except five have been carefully examined, immediately after death, by both the writer and his helpers. *Paraechinus* hedgehogs are very locally distributed and up to ten years ago they were unknown here. Our recent findings (Wassif, 1944, 1954, 1955, Wassif and Hoogstraal, 1954, Hoogstraal and Wassif, 1955, Hoogstraal, Wassif and Kaiser, 1955) indicate that we still have much to learn about these animals.

In the hedgehog genus *Erinaceus*, the Algerian species *E. a. algirus* Duvernoy and Lereboullet, 1848, has been found infested in Tunisia. The Near Eastern subspecies, *E. europaeus transcaucasicus* Satunin, 1905, is infested in Palestine. It may be of some interest to note that 35 specimens of *E. europaeus concolor* Martin, 1838, which I examined in the lowlands and steppes of western and eastern Anatolia (Turkey) in 1953 and 1954 were infested only by *Rhipicephalus s. sanguineus* and *Ixodes* sp.

In Europe and Russia, ticks identified as *H. numidiana*, as *H. tauranica* Pospelova-Shtrom, or as *H. taurica* Posp.-Sht., have usually though not always been reported to be from hedgehogs.

It is obvious, from material we have seen, that hedgehogs are the chief hosts of the immature stages, but that desert rodents may also serve as hosts of these stages. The Nuttall collection contains 17 nymphs from two Algerian dipodil gerbils, *Gerbillus* (*Dipodillus*) *campestris* subsp., and in Egypt we have found nymphs on the same and on a different species of dipodil gerbil, as well as on a dormouse, *Eliomys quercinus* subsp., and on a jird, *Meriones s. shawi*. As already noted, 17 larvae (one reared to nymph) and over 200 nymphs have been found on the Egyptian long-eared hedgehog, *H. auritus aegyptius*.

DISTRIBUTION: *H. erinacei* ranges along the Mediterranean littoral, and some distance inland, from Algeria to Egypt, and possibly to Palestine. Other southeastern European and Russian records under various names may refer to *H. erinacei* or to closely related species or subspecies, the status of which is not now certain.

In Egypt, *H. erinacei* appears to be confined to the western coastal area, though subsequent collection may reveal that it also exists in coastal areas of the Nile Delta and of Sinai. We have examined so many hedgehogs elsewhere in Egypt that it can be said with little hesitation that this tick does not follow its chief host into the Nile Delta, the Nile Valley, or the Faiyum.

H. ernacci[1] (syn. *H. numidiana*) has been reported from Transvaal, South

[1] In January, 1955, while making my third search for Warburton's (1933) "*H. numidiana*" material from South Africa in British Museum (Natural History), I was pleasantly surprised to find it in a vial in a bottle of materials which Warburton obviously considered doubtful and in need of further study. The three pencil-written labels in the vial read as follows: (1) "172 *Haem.* ? sp." (2) "Bedford's *H. cooleyi* see Oct. Rep. Pretoria." (3) "*numidiana* if not *leachi*." Another label in ink reads "172." The specimens, a single male and female, are freshly molted, unusually pilose, and in excellent condition; they are definitely not *H. erinacei* (=*numidiana*) and appear to represent an undescribed species in the *hoodi* group. The enigma of the South African range and of the non-erinacid host of *H. erinacei* is thereby removed.

Africa, by Warburton (1933) and Cooley (1934) from a spring-hare, *Pedetes cafer*. As will be shown in a subsequent paper in this series (on hyrax parasites), this record is so questionable that it appears best to omit South Africa from the range of this tick. The specimens cannot now be located in the Rocky Mountain Laboratory, British Museum (Natural History), or Onderstepoort collections.

Biology and Life Cycle: In Egypt, we have found adults of both sexes on hedgehogs in most months of the year, during winter (January), spring (March to May), summer (June to October), and fall (November). Larvae have been found in April and May; nymphs mostly in March and a few in April, May, October, and January. This is suggestive of heavier egg laying during the spring than at other times of the year, and of about one and a half generations a year in nature.

Our rearing data for 20 nymphs on hosts collected on 22 April is as follows: Dropped from hosts between 28 April and 7 May, molted to adults between 15 May and 2 June. The time between dropping from the host and molting was from 16 to 27 days, average 23 days (at temperature of 58°F. to 86°F., average daily temperature from 65°F. to 73°F.).

Twenty females on the same hosts on 22 April dropped from the animals between 11 May and 18 May (19 to 26 days) and commenced egg laying between 22 May and 28 May (7 to 11 days after dropping from host). Larvae hatched 17 June to 30 June (26 to 33 days—average 30 days—after eggs laid) (temperature and humidity as above). The considerable length of time that females remained on the host, at least 19 to 26 days after the host was collected in the field, is noteworthy. Laboratory-reared larvae did not feed on laboratory animals or hedgehogs. Attempts to obtain larval feeding will be made in the future.

The Egyptian long-eared hedgehog's range is continuous from the Mediterranean littoral through the Nile Delta and Nile Valley, only as far south as the Faiyum (Figure 35). In this area, it is everywhere common. The tick, however, has not followed its host beyond the littoral area, indicating that the parasite requires different factors for survival than does its host. The most suggestive factor is climatic. The most outstanding climatic feature of the Egyptian littoral is its relative humidity, which is considerably higher than that of inland Egypt.

It might be added that we are perplexed over why the very common Egyptian long-eared hedgehog does not extend its range further south in the Nile Valley than the Faiyum. No recognizable barriers or restricting factors exist at this level of the Nile Valley.

Position on Host: We find *H. erinacei* chiefly on the ears of hedgehogs, rarely among the soft hairs of the undersides. We have noted only two specimens from among the hosts' spines. *H. erinacei*, like *H. simplex* Neumann, 1897, of Madagascan spiny insectivores, which also feeds chiefly on the host's ears, is a morphologically unspecialized tick as compared with the Madagascan *elongata* group of ticks (Hoogstraal, 1953). The *elongata* group, comprising three species, is more commonly found among the insectivore hosts' spiny hairs, and each species in the *elongata* group has a remarkably specialized combination of coxal and trochantal spur arrangement, and of integumental stretching, to allow the tick to move and secure a hold on these large, hard hairs.

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SUMMARY

The male and female of *H. erinacei* are redescribed; the nymph, larva, and egg are described for the first time. In Egypt, the species is fairly common only on the Mediterranean littoral, though its host, the long-eared hedgehog, *Hemiechinus auritus aegyptius*, extends about 175 miles south of the coast in the Nile Valley. Larvae and nymphs parasitize hedgehogs, gerbils, dormice, and jirds. Breeding continues throughout the year, with a heavy springtime peak; one and a half generations a year appear likely in nature. Females remain on the host for three weeks or longer. Unspecialized for moving among the hosts' spiny hairs, adults usually feed on the ears.

A recent proposal for separating North African from Palestinian-Russian populations by the criterion of coxal hair numbers and lengths is shown to be one which must be very cautiously used due to wear and tear of these hairs in life and after death. North African and possibly Palestinian populations are considered to be all *H. erinacei* Pavesi, 1884. Larger series of specimens from North-West Africa and Palestine are necessary for the final evaluation of this criterion. The relation of this species to *H. taurica* Pospelova-Shtrom, of U.S.S.R. is uncertain.

ADDENDUM

Through the kindness of Dr. George Anastos of the University of Maryland and Dr. S. L. Tuxen of the University Zoological Museum of Copenhagen, I have been fortunate in being able to see two males and seven females, identified as *H. numidiana* (Anastos, in press) from Afghanistan. This material corresponds to *H. erinacei* Pavesi, 1884, as described above and greatly increases the known range of what is considered to be typical *H. erinacei*.

REFERENCES CITED

COLAS-BELCOUR, J. AND RAGEAU, J. 1951 Tiques de Tunisie: Ixodines. Arch. Inst. Pasteur, Maroc. **4**: 360–367.

COOLEY, R. A. 1934 A search for tick parasites in South Africa. Onderstepoort J. Vet. Sci. and Animal Indust. **3**: 23–42.

FELDMAN-MUHSAM, B. 1951 A note on East Mediterranean species of the genus *Haemaphysalis*. Bull. Res. Council Israel **1**: 96–107.

———1953 On the identity of *Haemaphysalis erinacei*, and *H. taurica*. Bull. Res. Council Israel **2**: 372–378.

HOOGSTRAAL, H. 1953 Ticks (Ixodoidea) of the Malagasy faunal region (excepting the Seychelles). Their origins and host-relationships; with descriptions of five new *Haemaphysalis* species. Bull. Mus. Comp. Zool. **111** (2): 37–113.

———1954 A preliminary, annotated list of ticks (Ixodoidea) of the Anglo-Egyptian Sudan. J. Parasit. **40**: 304–310.

HOOGSTRAAL, H. AND WASSIF, K. 1955 New mammal records from the Western Desert of Egypt, with notes on ectoparasites. (in press).

———, ———, AND KAISER, M. N. 1955 Results of the NAMRU-3 Southeastern Egypt Expedition, 1954: itinerary, biological and ecological observations, mammals and their ectoparasites. (in press).

NEUMANN, L. G. 1897 Revision de la famille des ixodidés. 2e mémoire. Mém. Soc. Zool. Fr. **10**: 324–420.

———1905 Notes sur les ixodidés, II. Arch. Parasitol., Paris **9**: 225–241 (1904).

PAVESI, P. 1884 Materiali per lo studio della fauna tunisina. II. Aracnidi. Ann. Mus. Stor. Nat. Genova **20**: 483–485.

WARBURTON, C. 1933 On five new species of ticks (Arachnida, Ixodoidea). *Ixodes petauristae, I. ampullaceus, Dermacentor imitans, Amblyomma laticaudae* and *Aponomma draconis*, with notes on three previously described species, *Ornithodorus franchinii* Tonelli-Rondelli, *Haemaphysalis cooleyi* Bedford, and *Rhipicephalus maculatus* Neumann. Parasitology **24**: 558–568.

WASSIF, K. 1944 On the occurrence of *Paracchinus dorsalis* (Anderson and De Winton) in South Sinai, with a note on the osteology of the animal. Bull. Fac. Sci. Egypt. Univ. **25**: 203–211.

———1954 On the occurrence of hedgehogs of the genus *Paracchinus* in the El Tahreer Province of Egypt. Bull. Zool. Soc. Egypt **11**: 40–47 (1953).

———1955 Results of the NAMRU-3 Southeastern Egypt Expedition, 1954: mammalian taxonomy. (in press).

WASSIF, K. AND HOOGSTRAAL, H. 1954 The mammals of South Sinai, Egypt. Proc. Egypt. Acad. Sci. **9**: 63–79 (1953).

AGE OF CHICKENS AND THE RATE OF MATURATION OF *ASCARIDIA GALLI*

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Ackert (1931) showed that following an infection approximately 50 days were required for *Ascaridia galli* to develop to sexual maturity and to produce eggs. It has been difficult, however, to correlate this finding with the consistent verbal reports from those making necropsy examinations of chickens raised as broilers. These reports stated that four-week old chickens harbored *A. galli* which were three or more inches long, indicating that the worms were mature or very nearly so. If we assume that the chickens began to acquire the infection the day they were placed in the poultry house, the field observations indicate that the infection matures much more rapidly than was indicated by Ackert.

The report of Tugwell and Ackert (1952), showing that the tissue phase of the life cycle of *A. galli* may occur as early as the first day of infection and that the larvae may leave the intestinal wall on the second day of infection, seemed to correlate with the field reports of early maturing worms. Therefore, it was decided to check on the time required for *A. galli* to mature as measured by the occurrence of eggs in the chicken droppings. The results of this study are the subject of this report.

All of the birds used in this work were raised in batteries. Chickens two months old and older were maintained in cages large enough to hold three or four birds. The results presented in the table are therefore obtained from a composite fecal sample and are not representative of individual birds. The single infection administered to the birds was the only infection available to them.

Chickens of different breeds, 12 to 16 days, 25 to 32 days, 62 days and 90 days old, were each administered an infective dosage of embryonated ova by pipetting the ova directly into the crop. The number of ova given to each bird varied from 386 to 950.

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Examination of the fecal material was made by the salt flotation method. The examinations were started 24 or 25 days after infection and were made on alternate days. The finding of a single ascarid egg was not considered to be proof of a mature infection.

As no ova were found previous to 30 days after infection, the results of the earlier examinations are not included in Table I. Examination of the table shows that in chickens infected up to and including 32 days of age, ova were consistently found the 30th to 33rd post-infection day. The 62 day old birds passed ova consistently from the 35th day after infection onward. In one case the birds infected at 90 days of age passed the first ova on the 50th post-infection day, while in the other case no ova were found at this time. When the latter birds were killed, the female worms recovered were immature.

Although this portion of the data needs to be substantiated, it appears that in the two month old bird, maturation of the worm requires a slightly longer time than in younger birds.

In order to prove the viability of the ova passed on the 31st post-infection day,

TABLE I.—*Showing the day after infection when* Ascaridia galli *ova appeared in chickens infected at different ages*

Age when infected, (days)	Number ova given	Days after infection ova were found*							
		30	31	32	33	34	35	39	50
12	422	+	0	+	0	0	0	0	0
16	386	0	+	0	–	0	+	0	0
25	386	0	–	0	+	0	–	0	0
26	386	0	+	0	+	0	0	0	0
26	772	0	+	0	+	0	0	0	0
31	633	–	0	+	0	0	0	0	0
32	450	0	0	+	+	0	0	0	0
62	950	–	0	–	0	0	+	+	0
90	832	–	0	–	0	–	0	–	+
90	622	–	0	0	0	0	–	–	**

* 0, no examination; –, negative for ova; +, positive for ova.
** Immature female worms found.

a chicken harboring these worms was killed and the ova from the worms were cultured. These ova developed normally and were infective to other chickens.

These results confirm the consistent field reports and show that in birds less than three months old the worms mature considerably more rapidly than in older birds. The more rapid development of the worms is of considerable significance in raising broiler chickens whose life span is about ten weeks. It is obvious that the earlier maturing of the worm will increase the contamination of the litter and hence increase the potential infection of the chickens. The increased potential infection would occur at the time the grower is trying to put a "finish" on the bird.

Ackert, Edgar, and Frick (1939), postulated a relationship between the number of goblet cells in the duodenum and the age resistance of the chick to this nematode. Their data indicated that the number of goblet cells found in a three month old bird was very nearly the same as that found in an adult chicken. The question is raised whether this relationship is applicable to the lengthening of the time required for this nematode to attain sexual maturity.

SUMMARY AND CONCLUSIONS

The rate at which *Ascaridia galli* attain sexual maturity, as indicated by the recovery of viable ova from the feces, was studied in chickens ranging from 12 to

90 days old. In chickens infected when less than 90 days old, viable ova were found 30 to 35 days after infection. In chickens infected when 90 days old, no mature ova were found previous to 50 days after infection. The more rapid development of the nematode in the younger chicken has considerable significance for the chicken broiler industry.

BIBLIOGRAPHY

ACKERT, J. E. 1931 The morphology and life history of the fowl nematode, *Ascaridia lineata* (Schneider). Parasitology **23**: 360–379.

ACKERT, J. E., EDGAR, S. A., AND FRICK, L. P. 1939 Goblet cells and age resistance of animals to parasitism. Tr. Am. Micro. Soc. **58**: 81–89.

TIDWELL, R. L. AND ACKERT, J. E. 1952 On the tissue phase of the life cycle of the fowl nematode *Ascaridia galli* (Schrank). J. Parasit. **38**: 277–288.

DEVELOPMENT OF *SCHISTOSOMA MANSONI* IN THE PERITONEAL CAVITY OF MICE[1]

DONALD V. MOORE[2] AND HENRY E. MELENEY[3]

The investigations of Faust and Meleney (1924) and Faust, Jones and Hoffman (1934) have shown that following percutaneous exposure of various mammalian hosts to cercariae the growth and development of *Schistosoma japonicum* and *Schistosoma mansoni* is dependent on the ingestion of portal blood by the young worms. Yolles, Moore and Meleney (1949) studying the post-cercarial development of *S. mansoni* in rabbits and hamsters following intraperitoneal and percutaneous routes of infection, found that in rabbits infected by the intraperitoneal injection of cercariae, worms could be recovered from the peritoneal cavity as late as 9 weeks after infection. However, none of these worms had advanced beyond the *mu* stage of development. In hamsters infected intraperitoneally, worms were found in the peritoneal cavity for 10 days after infection, but no further observations were made on hamsters. Once the schistosomulae reached the circulation from the peritoneal cavity their continued growth and development seemed to be dependent upon ingestion of portal blood.

While conducting certain chemotherapeutic experiments using mice which had been infected by intraperitoneal injection of *S. mansoni* cercariae, small worms were observed lying among the loops of the intestine in the peritoneal cavity of one animal. Flushing of the peritoneal cavity with a strong stream of citrated saline, prior to the ligation or severing of any blood vessels, revealed 25 to 30 small *S. mansoni*. The same procedure was followed at the autopsy of the remaining mice in this series and all of the mice were found to be harboring worms in the peritoneal cavity. The worms thus recovered were considerably smaller than those recovered from the mesenteric circulation and they had no hematin in the intestinal ceca, indicating that they had not fed on blood. Nevertheless, the sex of the worms

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from the peritoneal cavity was easily determined and small undeveloped genital organs could be seen. Many of these worms were in copula.

These observations led to this present investigation in which the development of worms remaining in the peritoneal cavity of mice is compared with that of worms which complete their development in the portal circulation. From our previous studies (Yolles *et al.*, 1949) we knew that worms persisted in the peritoneal cavity of hamsters for at least 10 days. As an adjunct to this study on mice, observations on hamsters were extended beyond 10 days.

METHODS

Cercariae were obtained from pooled groups of laboratory reared and infected *Australorbis glabratus*. Cercarial counts were made with the syringe and needle combination which was to be used for the injection of the animal according to the method described by Moore, Yolles and Meleney (1949). Albino mice (CF strain) were injected with 500 cercariae each and hamsters with 300 cercariae each.

The autopsy procedure was the same as that described by Yolles *et al.* (1949) with the necessary adaptations for smaller animals. The areas examined for schistosomes were peritoneal cavity, right and left heart, lungs, liver, mesenteric-portal circulation, lower extremity circulation and retroperitoneal lymph node. All washings and perfusates were collected in 15 ml. conical centrifuge tubes. The worms were concentrated by centrifugation. Perfusates containing blood were laked in distilled water and recentrifuged. The resulting sediment was examined in a watch glass with the aid of a dissecting microscope. The worms were counted and classified according to stage of development. In classifying stages of development we used the Greek letter stages originally described for *S. japonicum* by Faust and Meleney (1924) and later adapted to *S. mansoni* by Faust *et al.* (1934). Due to certain discrepancies in the designation of stages between these two groups of authors we used the criteria given by Faust, Jones and Hoffman (1934) though we combined the *alpha-beta* stages. Their criteria are quite satisfactory for the early stages but difficult to apply to the later stages of development in this particular study.

Measurements of worms were made from stained toto-mounts of the worms according to the method described by Moore, Yolles and Meleney (1954).

One mouse was autopsied each day for the first 10 days after infection and thereafter at 2-week intervals from 2 through 12 weeks. Hamsters were autopsied at 2-week intervals from 2 through 12 weeks.

RESULTS

MICE—The results of observations on worms recovered from mice are presented in Tables 1 and 2. Evidence of escape of some worms from the peritoneal cavity is seen in the mouse autopsied one day after infection when one worm in the *alpha-beta* stage was recovered from the lung (Table 1). In the mice autopsied on the second and third days no worms could be found outside of the peritoneal cavity. In the mice examined on days 4 through 10 from 3 to 10% of the worms recovered were extraperitoneal. On the fourth day worms in the *alpha-beta* stages were recovered from the heart and lungs indicating that they had just arrived from the peritoneal cavity. In the same animal worms in the *epsilon* and *zeta* stages,

which had probably completed their migration through the lungs, were recovered from the liver and mesenteric veins.

Fourteen days after infection the worms recovered from the heart and lungs were in the *alpha-beta, gamma* or *delta* stage. Twenty-eight days after infection a few worms were found in the heart and lungs, all in the *omicron* stage. Although Olivier (1952) has shown that following percutaneous infection of mice with *S. mansoni* the worms tend to pass through the lungs slowly resulting in an accumulation in the lungs, it is felt that the presence of *alpha-beta, gamma* or *delta* stages in the lungs of our 14 day animal probably indicates recent arrivals from the peritoneal cavity. The presence of the more advanced *omicron* stage in the lungs of the 28-day animal may represent the retarded passage of worms through the lungs. After 28 days no more worms were recovered from the heart and lungs. Thus we may assume that at some time between 14 and 28 days after infection no more worms escaped from the peritoneal cavity. This is in agreement with the

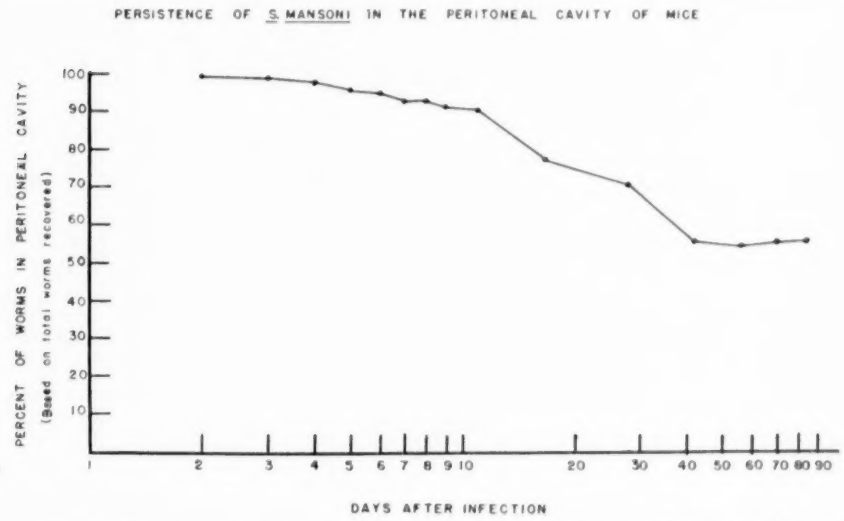

FIG. 1. Persistence of *S. mansoni* in the peritoneal cavity of mice. (Points plotted represent 3-point moving averages.)

observations of Faust *et al.* (1934) that the lungs of their animals (rabbits and monkeys infected percutaneously), were free of worms by the 20th day; and it indicates that in spite of an abnormal method of introduction of the cercariae the post-cercarial migration proceeded in a normal manner.

From 28 through 84 days essentially all of the extraperitoneal worms were recovered in the perfusates of the liver and mesenteries. Prior to 42 days more worms were recovered from the liver, as was expected. Subsequent to the 42nd day more worms were found in the mesenteric vessels.

The percentages of recovered worms found in the peritoneal cavity of the mice are presented in Figure 1. From Figure 1 and Table 1 it is evident that in mice infected by the intraperitoneal route many of the worms do not escape from this location. During the first 14 days 86.8 to 100% of the worms recovered were

TABLE 1.—*Worm recovery by location after intraperitoneal injection of 500* S. mansoni *cercariae into mice*

Days after infection	1	2	3	4	5	6	7	8	9	10	14	28	42	56	70	84
Location	Number of worms recovered by location															
Peritoneal Cavity	279	85	160	179	276	111	132	127	141	166	92	67	97	50	28	129
Retroperitoneal lymph node					2											
Right heart				1		4					1					
Lungs	1			2		3	3	3	4	3	3	2				
Left heart				1	1					3		1				
Lower extremities																
Liver				1	5		5	10	3	13	5	32	20	22	8	14
Mesenteric veins				1	1			2	1		5	23	19	46	20	25
Total	280	85	160	185	285	118	140	142	149	185	106	125	136	118	56	168
Extraperitoneal	1			6	9	7	8	15	8	19	14	58	39	68	28	39
Location	Percentage of total worms recovered															
Peritoneal cavity	99.6	100	100	97.0	96.8	94.1	94.3	89.4	94.6	89.8	86.8	53.6	71.3	42.4	50.0	76.7
Extraperitoneal locations	0.4			3.0	3.2	5.9	5.7	10.6	5.4	10.2	13.2	46.4	28.7	57.6	50.0	23.3
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

TABLE 2.—*Distribution of developmental stages of peritoneal (P) and extraperitoneal (EP) worms in mice after intraperitoneal injection of 500* S. mansoni *cercariae*

Stage of development	Days after infection																															
	1		2		3		4		5		6		7		8		9		10		14		28		42		56		70		84	
	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP	P	EP
α & β	279	1	83		156		29	1	31		21	1	54	4	6		3		14		12	1										
γ			2		4		114	4	225	1	32		34	1	21		6	2	16	4	5	1										
δ							35		20	6	32	3	29	1	31	5	27	3	27		9	1										
ε							1			1	11	2	6	2	36	4	54	1	29	5	12											
ζ								1		1	10		6		30	4	47	1	29	3	1	1										
η											5	1	3		3	1	4	1	29	2	8											
ι																1			14	4	7											
κ																			8	1	8	1										
λ																					10	2	10	1								
μ																					10	4	5	1	1							
ν																					10	3	7	5	1							
ξ																							12	2	2							
ο																							15	2	1	1						
π																							10	6	5						2	
ρ																							7	1	12						10	
σ																								9	14		6				27	
τ																							1	4	18		8		6		35	
υ																								8	20		15	1	8		36	
φ																								18	23		21	9	14	2	19	
χ																								1				5		2		8
ψ																										3		4		4		4
ω																										10				11		7
																										25		49		9		20

found in the peritoneal cavity. From 28 through 84 days, during which time the migration of worms out of the peritoneal cavity had apparently ceased, 42.4 to 76.7% (average 58.8%) of the worms recovered were still found in this location. There were only 2 animals in the series (56 and 70 days) in which the number of extraperitoneal worms exceeded or equalled the number of intraperitoneal worms. It is conceivable that some of the worms which did not escape from the peritoneal cavity may have been destroyed *in situ* although there was no evidence to support this assumption. All worms recovered from the mice, irrespective of location, were alive.

The number of extraperitoneal and intraperitoneal worms according to stage of development from 1 through 84 days is given in Table 2. A graphic representation of the growth of the extraperitoneal and intraperitoneal worms on the basis of the most advanced stage of development found at each time interval is presented in Figure 2. The points plotted on the graph represent three point moving averages of the most advanced stage seen.

During the first 14 days after infection we observed no essential difference in the growth and development of the worms from the peritoneal cavity and those from the extraperitoneal locations. It appears that the environment within the host exerts little influence on the growth of the worms during these early stages. On the 14th day the range of developmental stages for both intraperitoneal and extraperitoneal worms was from *alpha-beta* to *lambda* with no appreciable concentration in any one stage.

At 28 days the worms from the peritoneal cavity ranged from *kappa* to *sigma* and at the same time the extraperitoneal worms ranged from *kappa* to *phi*. The majority of the extraperitoneal worms were more advanced in development than the intraperitoneal worms, with most of them being in the *pi* to *phi* stages while the majority of the intraperitoneal worms were in the *kappa* to *pi* stages.

Classification of specific stages of development for the worms recovered from 42 through 84 days was difficult since the main differential criteria for the more advanced stages are length and progress of development of genital organs. These criteria are extremely variable, the variations being dependent, among other things, upon environmental conditions within the mammalian host. In view of these difficulties it must be recognized that the comparison of the development of the peritoneal and extraperitoneal worms, based solely on the classification of the worms according to stage, will not be as accurate as the comparison prior to 42 days. However, in spite of the difficulties it is felt that our classification of stages from 42 through 84 days tends to illustrate the comparative trend of development of both extraperitoneal and peritoneal worms.

At 42 days it was apparent that the extraperitoneal worms were considerably more advanced than the peritoneal worms. In this particular animal all except one extraperitoneal worm had reached sexual maturity (*chi* to *omega*) while no worms from the peritoneal cavity were mature at this time. The range of development of the peritoneal worms was *lambda* to *upsilon* with the majority of the worms falling in the range from *pi* to *upsilon*.

The worms from the animals sacrificed at 56 and 70 days were in similar developmental stages. The intraperitoneal worms ranged from *rho* to *upsilon* with a majority classified as *tau* and *upsilon*. The extraperitoneal worms were classified as ranging from *tau* to *omega*, the majority being sexually mature. (*chi* to *omega*).

The classification of some of the worms in the stages *tau* through *phi* may be attributed to the fact that these animals harbored excess male worms which were slightly smaller than the males paired with females.

The peritoneal worms from the animal autopsied 84 days after infection ranged from *omicron* to *upsilon*. Many of these worms appeared to be less advanced than the peritoneal worms from the animals autopsied at 63 and 70 days. This difference

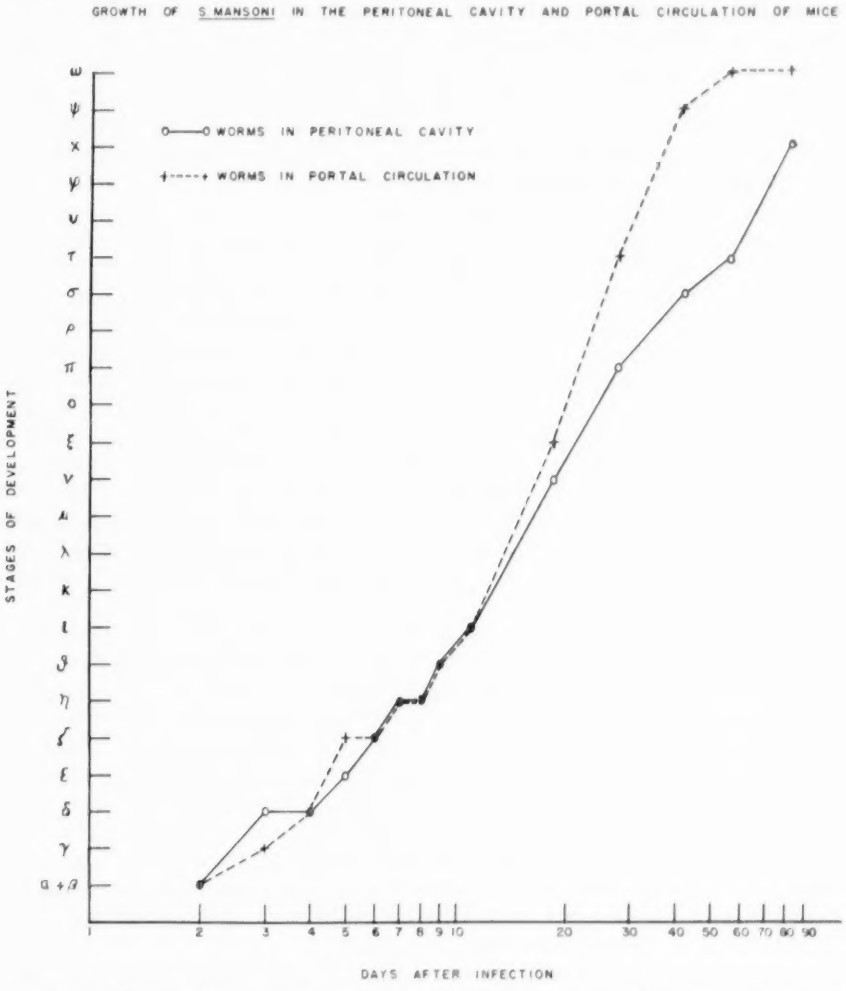

FIG. 2. Growth of *S. mansoni* in the peritoneal cavity and mesenteric-portal circulation of mice. Points plotted represent 3-point moving averages of most advanced stage.

in development might be attributed to differences between individual mice or to difficulties encountered in obtaining uniform classification of the more advanced stages. All of the worms from the extraperitoneal locations were sexually mature but differences in size caused them to be classified as ranging from *phi* to *omega*.

The above classification of stages of development was based solely on living

worms. Since a better comparision of final development can be made from a study of the genital organs in stained specimens a second series of mice was studied in which the worms were fixed, stained and mounted as toto-mounts. In this series the animals were autopsied at 2 week intervals from 4 through 28 weeks (28–168 days). This second series confirmed our previous observations of continued existence of worms in the peritoneal cavity of mice. Data obtained from this series shows that from 28 through 168 days an average of 67% (range 32–85%) of the total worms recovered were found in the peritoneal cavity. Thus worms which are unable to escape from the peritoneal cavity after their introduction there as cercariae are capable of surviving in this abnormal location for at least 168 days.

The sexual development of the males and females recovered from the mesenteric portal vessels corresponded with that which was reported previously in connection with another study, Moore *et al.* (1954). When the sexes are balanced all of the worms are usually sexually mature by 49 days.

The sexual development of the males and females from the peritoneal cavity presented certain differences from that in worms which completed their development in the normal locations. At 28 days it was easy to differentiate the sex of the worms recovered from the peritoneal cavity. The males had not yet attained maturity; the testes were small but the testicular cells did show evidence of considerable mitotic activity. These males averaged about 1 mm. in length while those from the mesenteric-portal circulation averaged 4.5 mm. in length. The females from the peritoneal cavity were immature and averaged 1 mm.; in the same animal the females from the mesenteric-portal circulation averaged 3.5 mm. in length.

Eleven of 32 male worms from the peritoneal cavity at 42 days after infection had spermatozoa in the seminal vesicle. These males averaged 4 mm. in length while those from the extra-peritoneal locations in the same animal averaged 7 mm. in length. The female worms from the peritoneal cavity averaged 2 mm. in length and were sexually immature. The ovary appeared to be in the same stage of development as that of a female from a unisexual infection as described by Moore, *et al.* (1954). The females from the extraperitoneal locations in the same animal averaged 8 mm. in length and were either sexually mature or nearly so.

All but 12 of 34 male worms from the peritoneal cavity at 56 days after infection had spermatozoa in the seminal vesicle. The average length was the same as that of the 42-day old males. There was essentially no change in development or length of the males from the extra-peritoneal locations. Six of 15 females from the peritoneal cavity of this animal had fully or partially developed, functioning vitellaria, and spermatozoa in the fertilization chamber of the oviduct. The ovary in these worms was small but the ovarian cells showed evidence of at least partial development. No eggs were seen in the oötypes of these females. The remaining females showed no indication of sexual maturation.

At 70 days there was little change to be seen in the males from the peritoneal cavity. The females from the peritoneal cavity exhibited even less development than the females from the 56-day animal. This lack of development of the females may be due to differences in worms and/or variations between mice.

The greatest number of female worms from the peritoneal cavity with evidence of sexual maturation was seen in the 84-day animal. Of the 59 females examined 58 showed quite extensive vitelline development, and the majority had vitelline

material in the vitelline duct. Ten of these worms had an egg in the oötype and spermatozoa in the fertilization chamber. Although the ovary remained small in these worms many showed either mature ova in the ovary or evidence of maturation of the ova. These females averaged 3 mm. in length, which was no increase over previous females from the peritoneal cavity. All of the male worms from the peritoneal cavity had spermatozoa in the seminal vesicle.

From 98 through 168 days there was no significant change in the worms from the peritoneal cavity. The male worms averaged 3.5 mm. in length and all had spermatozoa in the seminal vesicle. Wide variations were seen in the female worms. A great majority of the females remained sexually immature. All stages in the development of vitellaria were seen. Only a few females had developed to the point of producing eggs, but the ovary in a number of females showed evidence of partial development of ova. In spite of the fact that all of the male worms developed spermatozoa and males were consistently found in copula with females only a few inseminated females were observed. The ovary was measured in several worms which either had an egg in the oötype or partial development of ova. In these worms the ovary averaged 0.17 mm. long by 0.04 mm. maximum width. The ovary of the sexually mature females from extraperitoneal locations averaged 0.27 mm. by 0.08 mm. The females from the peritoneal cavity averaged 3.0 mm. in length while fully developed females from the extraperitoneal locations averaged 7.4 mm. in length.

In several instances eggs containing miracidia were recovered from the washings of the peritoneal cavity. These eggs were usually encased in mesenchyme cells.

HAMSTERS—From previous studies (Yolles *et al.* 1949) we had determined that when hamsters were infected by the intraperitoneal injection of cercariae some worms remained in the peritoneal cavity for at least 10 days. During the first 10 days an average of 52% (range 9 to 98%) of the total worms recovered were found in the peritoneal cavity. The most advanced stage found in the peritoneal cavity during the first 10 days was *gamma*. Since Yolles *et al.* (1949) did not carry their observations beyond 10 days we extended the observations on hamsters in this present study by examining hamsters which had been given intraperitoneal injections of 300 cercariae each. The animals were sacrificed at 2-week intervals from 2 through 12 weeks after infection. In the 10-day animal examined by Yolles *et al.* (1949) 18% of the worms recovered were from the peritoneal cavity. In the present series beginning at 14 days we found only 1% of the worms in the peritoneal cavity. These were in the *gamma* stage. No worms were recovered from the peritoneal cavity of any of the other hamsters examined 28 through 84 days. Thus it appears that in the hamster nearly all of the worms escape from the peritoneal cavity by 14 days and none persist in this location for any extended period.

DISCUSSION AND SUMMARY

Yolles *et al.* (1949) concluded that when cercariae of *S. mansoni* are injected into the peritoneal cavity of the rabbit there is a high initial mortality. However, some worms persisted in this location for at least 63 days and underwent partial development. The worms remaining in the peritoneal cavity did not develop beyond the *epsilon* or *zeta* stage. Worms which succeeded in escaping from the peri-

toneal cavity of the rabbit were slow to reach maturity in the mesenteric portal vessels when compared with the development in a more suitable host such as the hamster.

Mice and hamsters are about equally satisfactory as hosts for *S. mansoni*, on the basis of percentage of worm recovery, length of time required for the maturation of the worms and passage of eggs in the feces. The present study has shown that when cercariae are injected into the peritoneal cavity of mice and hamsters there is a marked difference in the response of the two hosts. In mice over 50% of the total worms recovered can be found in the peritoneal cavity for at least 168 days. In contrast to the worms found in the peritoneal cavity of rabbits the worms remaining in the peritoneal cavity of mice continue their growth and development in this abnormal location, without benefit of blood, and at least some of the female worms are able to complete their sexual maturation and produce fertile eggs in which miracidia develop. The females in the peritoneal cavity are considerably stunted, being about 50% shorter than mature females from the mesenteric portal system. The worms which manage to escape from the peritoneal cavity complete their migration to the mesenteric portal system and complete their development there without exhibiting any evidence of adverse effects as a result of their sojourn in the peritoneal cavity.

For the first 14 days the rate of development of the peritoneal worms is essentially the same as that of worms from the extraperitoneal locations in the same animal. After 14 days the extraperitoneal worms develop more rapidly, the majority reaching sexual maturity by 42 days while sexual maturity is not reached by the females in the peritoneal cavity until some time between 70 and 84 days. Some male worms from the peritoneal cavity reach sexual maturity as early as 42 days and by 56 days nearly all males are mature. In spite of the earlier maturation of the males in the peritoneal cavity, their continuation in this location and the fact that mature males were nearly always found in copula with the females, only a very few females became inseminated. Thus it would appear that conditions in the peritoneal cavity are not satisfactory for the consistent completion of their development. It is possible that one of the missing requirements is blood for the worms to feed upon.

In the hamster the majority of the worms migrate from the peritoneal cavity by 14 days, and none was found in this location after 28 days, in contrast to the findings in mice.

One practical application of the observations in mice recorded here is the recognition that in experiments in which mice are infected with *S. mansoni* by intraperitoneal injection of cercariae, perfusion of hepatic or mesenteric vessels for recovery of worms may yield worms which were not in those vessels but were on the peritoneal surface of the liver or intestines. This might yield confusing results if maturity of worms or their location were criteria for the interpretation of results of experiments.

References

Faust, E. C., Jones, C. A. and Hoffman, W. A. 1934 Studies on schistosomiasis mansoni in Puerto Rico. III. Biological studies. 2. The mammalian phase of the life cycle. Puerto Rico J. Pub. Health Trop. Med. **10**: 133–196 (English Text).

———, and Meleney, H. E. 1924 Studies on schistosomiasis japonica. Am. J. Hyg. Monogr. series No. 3. 339 pp.

MOORE, D. V., YOLLES, T. K. AND MELENEY, H. E. 1949 A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni*. J. Parasit. **35**: 156–170.

———, YOLLES, T. K. AND MELENEY, H. E. 1954 The relationship of male worms to the sexual development of female *Schistosoma mansoni*. J. Parasit. **40**: 166–185.

OLIVIER, L. 1952 A comparison of infections in mice with three species of schistosomes, *Schistosoma mansoni*, *Schistosoma japonicum* and *Schistosomatium douthitti*. Am. J. Hyg. **55**: 22–35.

YOLLES, T. K., MOORE, D. V. AND MELENEY, H. E. 1949 Post-cercarial development of *Schistosoma mansoni* in the rabbit and hamster after intraperitoneal and percutaneous infection. J. Parasit. **35**: 276–294.

THE IDENTITY OF *ORNITHODOROS SAVIGNYI* (AUDOUIN, 1827) AND *O. PAVIMENTOSUS* NEUMANN, 1901 (IXODOIDEA, ARGASIDAE)

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As a result of our interests in the identity, biology, and medical relations of African ticks, we have been concerned over the differentiation between two species often regarded as medically important, *Ornithodoros savignyi* (Audouin, 1827), and *O. pavimentosus* Neumann, 1901. The distributional range of *O. savignyi* includes many parts of continental Africa besides Arabia, India, and Ceylon. Although the range of *O. pavimentosus* is listed in reference works as only South-West Africa, various collections contain specimens from other parts of Africa identified as this species by different authorities.

HISTORICAL REVIEW

Neumann (1901) described *O. pavimentosus* from a single dry, apparently fully developed female from Namaqualand, South-West Africa, and gave as differences from *O. savignyi:* "Pattes, plus courtes que chez *O. savignyi* . . . par les granulations tégumentaires qui sont contigués, et par les deux derniers articles des pattes qui sont courts et à dents trés rapprochées." Later, Neumann (1911) wrote for *O. savignyi:* "5^{e} and 6^{e} articles des pattes, I, II and III a 3 dents ecartées plus hautes que longues," for *O. pavimentosus:* "aussi hautes que longues."

Dönitz (1906) compared additional South-West Africa specimens, collected by Dr. L. Schulze, with Neumann's type specimen of *O. pavimentosus*, and noted that in dry specimens the mammillae are flat and touching one another (i.e. Neumann's "contigués") so as to suggest Neumann's name of *pavimentosus*. Dönitz also observed that the tarsal humps of the flattened-mammillae specimens from South-West Africa were the same as those of *O. savignyi*. Later, Dönitz (1910)

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noted that the mammillae of *O. savignyi* may also be flat, hence this character is misleading. Having discarded mammillae and tarsal humps as diagnostic criteria, Dönitz (loc. cit.) advanced new features for differentiation: (1) in *O. pavimentosus*, mammillae show fine punctations under high magnification, but this is not true in *O. savignyi*, and (2) in *O. pavimentosus* only the deeper portions of the characteristic, *moubata-savignyi*, long muscular grooves are present and the shallow connecting grooves are absent.

We believe it reasonable to assume that both Neumann and Dönitz were discussing the same kind of tick. In January, 1949, one of us (G.T.) approached Dr. P. Schulze about this matter, and received the following reply, "Ich habe im Berlin Museum nur das Schulzische material (*pavimentosus*) (i.e. that studied by Dönitz) aus Namaqualand gesehen, und die Tiere schienen mir verschieden von *savignyi*. Auch das Hallersche Organ war etwas abweichend (siehe meine Arbeit No. 538). Auf alle Fälle steht die Form *savignyi* sehr nahe. Vielleicht kommon Stücken mit flachen Tuberkeln, aber gelegentlich als Mutation vor. Bei Hagenbeck fand ich eine ganze *moubata* Population auf Reptilien mit flachen Mammillen, die sonst auch in Hallerschen Organ mit *moubata* ubereinstimmen."

Diagnostic differences between Haller's organ might have been acceptable had it not been for subsequent work by Dr. F. Zumpt, a firm disciple of the late Dr. Schulze, who examined many tick species for Haller's organ, and told me (G.T.) that he had lost faith in this organ, as of diagnostic value.

The sum total, then, amounts to the fact that authorities who have examined material referrable to *O. pavimentosus* negated the value of one important criterion (flat, contiguous mammillae) and disagreed that there was an actual difference in the second (tarsi). Another character, Haller's organ, appears to be useless. Other characters, punctation of mammillae and integumental grooves were substituted.

EXAMINATION OF SPECIMENS

To satisfy ourselves concerning real differences between all characters proposed by Neumann and Dönitz, we have examined about 1000 specimens for these features. The material has come from South Africa (Postmasburg, Hay District), Sudan (Wad Medani, Wad Riya, Goz Regeb, Karora, Fasher, Khor Mashi, El Obeid, Bir Qwi Tiri, Um Bader, and Kosti), Egypt (El Arish on Sinai Peninsula, and various localities in Giza Province), Libya (Bengasi), and Arabia (Yemen, Ta'izz and Hodeida).

Almost every lot contains some specimens with hemispherical mammillae and some with flat mammillae. Many specimens throughout these lots have flat mammillae dorsocentrally and hemispherical ones laterally. The range of distance between flat mammillae and between hemispherical mammillae is variable in both conditions, both from specimen to specimen and for single specimens. In some they are widely separated, in a few almost contiguous. In some specimens small mammillae occur betwen large ones, in others they are absent.

To test Dönitz's character of fine punctation on mammillae, we have compared living individuals with dead, dry specimens and with alcohol-preserved specimens from the same colony. In preserved material, the mammilla surface appears minutely shagreened, in air-dried and in living specimens exceedingly minute punctations

are visible under high magnification. Shallow punctations are discernible as such on a certain number of very large preserved specimens.

We can find no noteworthy tarsal variation among this material. The presence or absence of shallow, connecting muscular grooves of the integument is variable in any series, due mainly to folding of the integument and degree of stretching due to engorgement, and possibly to the strength of internal muscular attachment and preservation of internal structures.

CONCLUSIONS

These observations amount to a reduction of all criteria proposed for differentiating between *O. pavimentosus* and *O. savignyi* to individual variations within the latter species. Indeed some of this variation appears to be not even biological but due rather to slightly different facies between living specimens and specimens preserved at a different age or degree of engorgement, or to be minute differences due to alcoholic preservation or dry preservation.

The only biological data published for *O. pavimentosus* are that it bites man resting under trees in the semideserts of South-West Africa, even during the day. *O. savignyi* has the same biting habits, and is found in similar ecological areas.

We therefore have no recourse but to consider *O. pavimentosus* Neumann, 1901 as a synonym of *O. savignyi* (Audouin, 1827), unless other distinguishing characters can be found.

SUMMARY

An investigation of characters proposed to differentiate *Ornithodoros savignyi* (Audouin, 1827), from *O. pavimentosus* Neumann, 1901, shows that these features amount merely to variations of *O. savignyi* apparently due to individual variation, age, degree of engorgement, and method of preservation. The name *O. pavimentosus* is therefore relegated to synonomy under *O. savignyi*.

REFERENCES

DÖNITZ, W. 1906 Über afrikanische Zecken. Sitzungsb. Gesellsch. Naurf. Fr. Berlin, (5), 143–148.

———1910 Die Zecken Südafrikas (In Schultze, Leonhard: Zool. u. Anthropol. Ergeb. einer Forschungsreise in westl. u. zentral. Südafrika **4**, 3 Lief). Denkschr. Med-Naturw. Gesellsch. Jena. **16**: 397–494 (pp. 27–124).

NEUMANN, L. G. 1901 Révision de la famille des ixodidés. 4e mémoire. Mém. Soc. Zool. France **14** (2–3): 249–372.

———1911 Ixodidae, xvi + 169 pp., Berlin (Das Tierreich, 26. Lief.)

THE LIFE-HISTORIES OF *ALLASSOGONOPORUS VESPERTILIONIS* MACY AND *ACANTHATRIUM OREGONENSE* MACY (TREMATODA: LECITHODENDRIIDAE)[1]

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Allassogonoporus vespertilionis Macy and *Acanthatrium oregonense* Macy are lecithodendriid trematodes parasitizing the small intestines of various species of bats. The first species was described by Macy (1940), and the second by him (1939). The life cycle of no species of either genus has been reported previously to this work.

Skrjabin (1915) found the metacercariae of *Lecithodendrium chilostomum*, an old, well-known species in caddis larvae of the genus *Phryganea*. Brown (1933) traced the metacercariae through the caddis larva, pupa, and imago. He found that they over-wintered in the larva and were encysted in the thoracic muscles of the imago.

This study traces the life history of a cercaria found in *Oxytrema silicula* (Gould), which proved to be *Acanthatrium oregonense*, and of a cercaria in *Fluminicola virens* (Lea), which was found to be *Allassogonoporus vespertilionis*, to determine the trematode species and the hosts involved in their life cycles. Originally the problem included only the cercaria found in *Oxytrema silicula*, but in the process of working out its life cycle, another closely connected life cycle was encountered. Within the caddis larvae were found two metacercariae which developed into adults when fed to a hamster, making it necessary to work out both cycles to be certain of either one. The cycles were traced from the cercaria to the adult, but no snails were experimentally infected.

New information relating to the esophagus and excretory bladder of the adult *Acanthatrium oregonense* has permitted emendation of the original description.

MATERIALS AND METHODS

Infected snails of the species *Oxytrema silicula* and *Fluminicola virens* were collected as needed from the upper Alsea River, near the Alsea fish hatchery 15 miles west of Philomath, Oregon, from Rock Creek, 2 miles west of Philomath, both in Benton County, and from the upper Yaquina River 6 miles from Summit, Oregon, in Lincoln County, throughout the period from September, 1953 to August, 1954. The snails were maintained in aquaria containing stream water, continuously aerated and kept at approximately 11° C. The desired infections were obtained by isolating the snails in water allowed to warm to room temperature for several hours to several days.

The search for the second intermediate host included examination of fish, copepods, and caddis fly larvae of the genus *Limnophilus*. The last harbored a metacercaria similar to the cercaria from *Oxytrema silicula*, but when the caddis

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larvae were fed to a hamster, two adult worms, *Allassogonoporus vespertilionis* and *Acanthatrium oregonense*, developed in the small intestine.[3] Closer examination of the caddis larvae revealed a second, encysted metacercaria. Uninfected caddis fly larvae of the genus *Limnophilus* were obtained from the Metolius River in Jefferson County, Oregon; and from Indian Ford in Deschutes County, 5 miles west of Sisters, Oregon, on the Santiam Highway. They were placed in bowls of stream water either with several *Oxytrema silicula* or *Fluminicola virens* discharging cercariae. When examined on the following day the *Limnophilus* larvae contained immature metacercariae which were recognized as those giving rise to *Acanthatrium oregonense* and *Allassogonoporus vespertilionis*.

Sexually mature trematodes belonging to these species were recovered from several bats, *Myotis lucifugus* (Le Conte), captured in an attic near Peoria, Oregon, in Linn County; and one mature *Allassogonoporus vespertilionis* was recovered from a single bat, *Eptesicus fuscus* (Beauvois), shot near Irish Bend, on the Willamette River, 12 miles south of Corvallis.

Measurements, observations, and drawings were made of living sporocysts, cercariae, and metacercariae stained with neutral red. The measurements of sporocysts, cercariae, and metacercariae were made with a measuring eye-piece. Adult flukes were fixed in an alcohol-formalin-acetic acid mixture without flattening, stained, and mounted for study. The drawings were made with the aid of a camera lucida or a microprojector. Details of anatomy were inserted free-hand. A pantograph was employed to bring the drawings of a series to the same scale to show relative size.

LIFE-HISTORY OF ALLASSOGONOPORUS VESPERTILIONIS MACY
(Figs. 6, 7, 8, & 9)

One sexually mature *Allassogonoporus vespertilionis* (Fig. 6) was recovered from the intestine of the bat *Eptesicus fuscus*, and several were recovered from the intestines of *Myotis lucifugus*. In both cases, however, the individuals were considerably smaller and contained fewer eggs than those which had developed in a hamster for three weeks, indicating the possibility that the infection had been acquired recently. One of 6 *E. fuscus* collected south of Corvallis, Oregon, had a single specimen of *A. vespertilionis* along with many other flukes of several species. All of the 10 *M. lucifugus* collected near Peoria, Oregon, contained *A. vespertilionis*. This species of fluke was originally discovered by Macy (1940) in *M. californicus caurinus*, collected near McMinnville, Oregon, in 1938. He found 70 specimens in that individual host. Several hundred specimens were collected from hamsters, experimentally infected during this study, indicating that the hamster is an excellent host for this worm.

The adult worm agrees in anatomy and size with data presented in Macy's original description.

Sporocysts. The daughter sporocysts (Fig. 7) from the digestive glands of *Fluminicola virens*, contained cercariae in varying stages of development. The sporocysts averaged 0.32 mm. long by 0.14 mm. wide, range 0.22 to 0.41 mm. long

[3] Dr. Ralph Macy of Reed College examined the material and confirmed the identification of the adult worms.

by 0.12 to 0.15 mm. wide. The number of mature cercariae within the sporocyst varied from 1 to 3, the larger sporocysts containing larger numbers.

Cercariae. The cercariae (Fig. 8) of *Allassogonoporus vespertilionis* belong to the cercariae virgulae group established by Lühe (1909). They are very poor swimmers, managing only with great difficulty and thrashing of tail to remain suspended in the water even momentarily. More often they settle to the bottom of the container, and creep about on their suckers in a caterpillar-like fashion. When stained with neutral red the cercariae become less active and more relaxed, although they are still not immobile. When contracted, they assume an ovoid shape with the tail folded in an accordion-like manner. A living semi-relaxed individual measures 0.16 mm. in total length, body length 0.102 mm. The extended tail is slender and longer than the body. Most often the tail is contracted, and then measures 0.04 to 0.06 mm. long. The tail readily falls off, and many tailless cercariae were seen creeping about. The oral sucker is 0.027 mm. long and 0.025 mm. wide. The cuticular surface is covered by minute spines which diminish in number and size posteriorly. The stylet measures 0.016 mm. long. It has a rounded protuberance on each side near the tip in either dorsal or ventral aspect. A side view shows the protuberances to be part of ring incomplete on the ventral surface, and that the tip is bent ventrally to make a crook. A conspicuous virgula (Fig. 8) which stains a brilliant red with neutral red vital dye was also found within the oral sucker. A small pharynx is present at the base of the oral sucker, and the esophagus and intestinal ceca could vaguely be made out extending around and dorsal to the ventral sucker. The latter is in the approximate center of the body, and slightly smaller than the oral sucker. Dorsal to, and on either side of the acetabulum, are three penetration glands which, like the virgula, stain a brilliant red. The glands are located in a longitutudinal series, the actual position varying with movements of the cercaria. The ducts of the penetration glands pass anteriorly and dorsally, and open to the exterior just anterior and dorsal to the oral sucker. At the posterior end of the body lies the excretory bladder, a thick-walled, simple vesicle, showing no indication of a bicornuate structure. The bladder is very flexible, changing shape as the cercaria moves. Its pulsations were frequently observed.

Contact of the cercariae with caddis fly larvae seemed to be merely by chance, no evidences of attraction by the larvae being observed. The cercariae crawled about on the surface of the caddis larvae, but actual penetration was never witnessed.

Metacercariae. The fully developed metacercariae (Fig. 9) are found encysted within the larval caddis fly among the fat globules of the body. The cyst is spherical, 0.142 mm. in diameter. The metacercaria much resembles the adult worm, except that reproductive structures are lacking. The cuticular surface is spinous, more thickly so at the anterior end, and thinning out to bareness at the posterior end. The spines are peculiar in that they resemble minute scales. The oral sucker is unarmed, having lost the stylet, also the virgula, and measures 0.043 mm. in diameter. The prepharynx is exceedingly short and membranous. The esophagus is thin-walled, and very short, expanding posteriorly into the thick-walled intestinal ceca. The bifurcation of the ceca makes a T-connection with the esophagus. The intestinal ceca extend from the bifurcation laterally and then rearward almost to the posterior end of the worm. Directly posterior to the cecal bifurcation lies the ventral sucker, 0.055 mm. in diameter. The most conspicuous structure present in the metacercaria is the simple, sac-like excretory bladder which occupies as much as half the

volume within the cyst. The excretory pore opening to the exterior seems to be closed because the excretory concretions found within the bladder do not pass through the pore into the interior of the cyst. The stem of the bladder is short. No flame cells were observed in the metacercaria because of the opacity of the vacuolated parenchyma of the worm.

Sexually mature adult worms were obtained from a hamster 6 days after feeding it infected caddis fly larvae. The infections were very heavy (100 or more flukes) from a feeding of 10 infected caddis fly larvae. Larvae, infected for 7 days with the cercariae of *Allassogonoporus vespertilionis*, were fed to a hamster, but no adult worms developed, indicating that maturation of the metacercaria of this species requires more than 7 days.

Eggs from the feces of the bat hosts must reach the water in which *Fluminicola virens* lives. The miracidium was not observed. The sporocysts observed by us presumably develop from a parent sporocyst which arose from a miracidium. The daughter sporocysts produce cercariae which emerge from the snail and penetrate into caddis fly larvae. Within the hemocoele they encyst and lie passively in any part of that cavity. The invading cercariae encysted immediately after entering the hemocoele of the caddis fly, but do not become infective within one week.

Incidence of infection was high in the bat, *Myotis lucifugus*, every individual taken being infected with many adult flukes in the small intestine. However, in a total of 6 bats of the species *Eptesicus fuscus* examined only one individual was found. This indicates differences in the insects taken by the two species of bats.

LIFE-HISTORY OF ACANTHATRIUM OREGONENSE MACY
(Figs. 1, 2, 3, 4, & 5)

Many sexually mature *Acanthatrium oregonense* (Fig. 1) along with other trematodes including *Allassogonoporus vespertilionis* were recovered from the intestines of the bat, *Myotis lucifugus*. A few specimens were collected from experimentally infected hamsters. *Acanthatrium oregonense* was originally found by Macy in the bats, *M. californicus caurinus* and *M. evotis evotis*, collected at Nelscott, Oregon.

The anatomy of the adult fluke agrees with the description given by Macy (1939) for the most part. Macy stated that the esophagus was apparently absent. In specimens studied by us an esophagus was observed. It describes a dorsal loop before joining the intestinal ceca which are located very close to the pharynx. The esophagus appears to be absent because it is doubled back dorsally, sometimes actually over the ceca and pharynx which lie adjacently in fixed specimens. The excretory bladder was not mentioned by Macy. It is very large, thin-walled, in the shape of a broad V, and occupies nearly all of the posterior half of the worm. These characters, obvious in living individuals, are difficult to see in preserved and stained specimens.

Sporocysts. No first generation sporocysts were seen, and the sporocysts (fig. 2) obtained from digestive glands of *Oxytrema silicula* contained cercariae in all stages of development. The sporocysts average 0.25 mm. long by 0.178 mm. wide, varying from 0.24–0.43 mm. long by 0.17–0.27 mm. wide. The number of mature cercariae within the sporocyst ranges from 1 to 6, the larger sporocysts containing the greater number of cercariae.

Cercariae. The cercaria (Fig. 3) belongs to the cercariae virgulae group

established by Lühe (1909). Superficially, this cercaria resembles that of *Allassogonoporus vespertilionis*, both in behavior and gross appearance. Both are poor swimmers, and usually creep about surfaces in a caterpillar-like fashion. Neutral red vital stain causes them to slow down for study in addition to differentiating structures by staining. Living, relaxed cercariae assume an oval shape with the accordian-like folding of the tail similar to that seen in cercariae of *A. vespertilionis*. A semi-contracted specimen measures 0.19 mm. long by 0.07 mm. wide. The extended tail is slender and naked, roughly 0.10 mm. long. The oral sucker has a diameter of 0.024 mm. The stylet, 0.014 mm. long, has a frontal appearance resembling that of cercaria of *A. vespertilionis* with two laterally placed, rounded protuberances near the tip. In side view the straight stylet tapers from the base to the pointed tip. The virgula, brilliantly staining in neutral red, occupies nearly three-fourths of the oral sucker. The ventral sucker, located 0.046 mm. from the anterior end of the worm, is nearly round and measures 0.012 mm. in diameter.

Laterally, anterior to the ventral sucker on either side are two, adjacently located, penetration glands. The neutral red dye stains them bright red, the unstained prominent nucleus forming a clear area in the center of each gland cell. The ducts from the gland cells open to the exterior just dorsal to the oral sucker. No traces of the ceca or excretory bladder were observed.

Although cercariae were not obviously attracted to the caddis fly larva, and no actual penetration was observed, newly penetrated cercariae were seen inside the host upon dissection.

Metacercariae. The metacercariae (Figs. 4 & 5) can be found moving about in the body fluids of the caddis fly larvae. This stage of *Acanthatrium oregonense* was never seen encysted. The stylet remains in the oral sucker of the young metacercaria. Intermediate stages of development of the metacercaria in the larval caddis were seen, ranging from newly penetrated ones to those which were infective.

The length of the living metacercaria is 0.32 mm., width 0.26 mm. The cuticle is smooth. In the infective form the stylet has disappeared. The diameter of the oral sucker is 0.055 mm. The pre-pharynx is absent or very short. The spherical pharynx leads into the esophagus which is about 0.045 mm. long. The ceca bifurcate a short distance in front of the ventral sucker, and are short and sac-like, in some worms directed obliquely posteriad, but in others forming right angles with the esophagus. The position of the ceca within the metacercaria depends upon movements by the individuals being studied. The ventral sucker with a diameter of 0.035 mm. is located in the approximate center of the body. It is a little smaller than the oral sucker. Behind the acetabulum and filling the posterior region is the large V-shaped excretory bladder (Fig. 5). Its walls are thick, cellular, and highly distensible. The bladder was frequently seen filling and emptying itself, discharging its contents to the exterior; however, no flame cells were ever seen. Probably they were obscured by the opacity of the parenchyma and body walls.

Mature worms were found in a hamster 6 days after it had been fed infected caddis fly larvae. Although many infective metacercariae were present in the caddis larvae the number of adults of *Acanthatrium oregonense* recovered from the hamster was always small. The ratio of *Allassogonoporus vespertilionis* to *Acanthatrium oregonense* in this host species was something over 50 to 1, although approximately equal numbers of metacercariae were judged to be present in the caddis fly larvae

that were fed. Evidently the hamster is a poor experimental host for the growth and development of *A. oregonense*. Metacercariae 7 days old and still retaining their stylets did not develop when fed to a hamster, indicating that the period required for maturation of this stage is more than a week.

Ova from the feces of the bat hosts must reach the water in which *Oxytrema silicula* lives. Neither the miracidium nor the first generation sporocyst were observed in this study. Probably the sporocyst develops from the miracidium and in turn produces daughter sporocysts that in turn produce cercariae which emerge from the snail and invade the hemacoele of the caddis fly larva of the genus *Limnophilus*. If this species follows the developmental pattern of *Lecithodendrium chilostomum*, encystment may follow pupation of the caddis fly, but has not been observed. In *Lecithodendrium chilostomum* encystment occurred in the developing thoracic muscles.

As stated above, Macy (1939) reported this fluke from two species of *Myotis*. To this host list is now added *M. lucifugus*, wherein the parasite was found commonly in the small intestine of every specimen examined.

SUMMARY

The major larval stages in the life histories of *Allassogonoporus vespertilionis* and *Acanthatrium oregonense*, lecithodendriid trematodes of bats, have been identified and described. Infective metacercariae of both species of trematodes were discovered in caddis fly larvae of the genus *Limnophilus* and were fed to the hamster under laboratory conditions. Adult trematodes of both species were recovered from the hamster and identified. Two groups of uninfected caddis fly larvae were experimentally infected with the cercariae of *Allassogonoporus vespertilionis* and *Acanthatrium oregonense*, respectively. Both species of experimentally produced metacercariae were recognized and identified as belonging to the same species which developed into the adult worms.

Sporocysts of *Acanthatrium oregonense* develop in the digestive glands of *Oxytrema silicula*, and those of *Allassogonoporus vespertilionis* similarly in *Fluminicola virens*. Virgulate xiphidiocercariae are produced by daughter sporocysts.

The cercariae penetrate caddis fly larvae of the genus *Limnophilus* and develop into metacercariae. Metacercariae of *Allassogonoporus vespertilionis* are encysted; whereas those of *Acanthatrium oregonense* are not.

All the stages in the two life cycles, that were seen, have been illustrated, and anatomical descriptions made.

REFERENCES CITED

BROWN, F. J. 1933 On the excretory system and life history of *Lecithodendrium chilostomum* (Mehl.) and other bat trematodes, with a note on the life history of *Dicrocoelium dendriticum* (Rudolphi). Parasitology **25**: 317–328.

LÜHE, M. 1909 Parasitische Plattwürmer. 1: Trematodes. *In* Die Süsswasserfauna Deutschlands (Brauer). Heft 17, IV + 217 pp.

MACY, R. W. 1939 A new trematode, *Acanthatrium oregonense* (Lecithodenriidae), from bats of the genus *Myotis*. Am. Midland Naturalist **22**: 640–641.

———1940 A new species of trematode, *Allassogonoporus vespertilionis* (Lecithodendriidae), from an Oregon bat, *Myotis californicus caurinus* Miller. Tr. Am. Micr. Soc. **50**: 48–51.

SKRJABIN, K. I. 1915 Contribution à la biologie d'un trématode *Lecithodendrium chilostomum* (Mehl., 1831). Compt. Rend. Soc. Biol., Paris **78**: 751–754.

EXPLANATION OF PLATE

All scales represent 0.1 mm.

FIG. 1. Adult *Acanthatrium oregonense* from the intestine of the hamster.

FIG. 2. Sporocyst of *Acanthatrium oregonense* from *Oxytrema silicula* showing 5 mature and 2 immature cercariae.

FIG. 3. Cercaria of *Acanthatrium oregonense*.

FIG. 4. Immature metacercaria of *Acanthatrium oregonense* with stylet.

FIG. 5. Mature metacercaria of *Acanthatrium oregonense*.

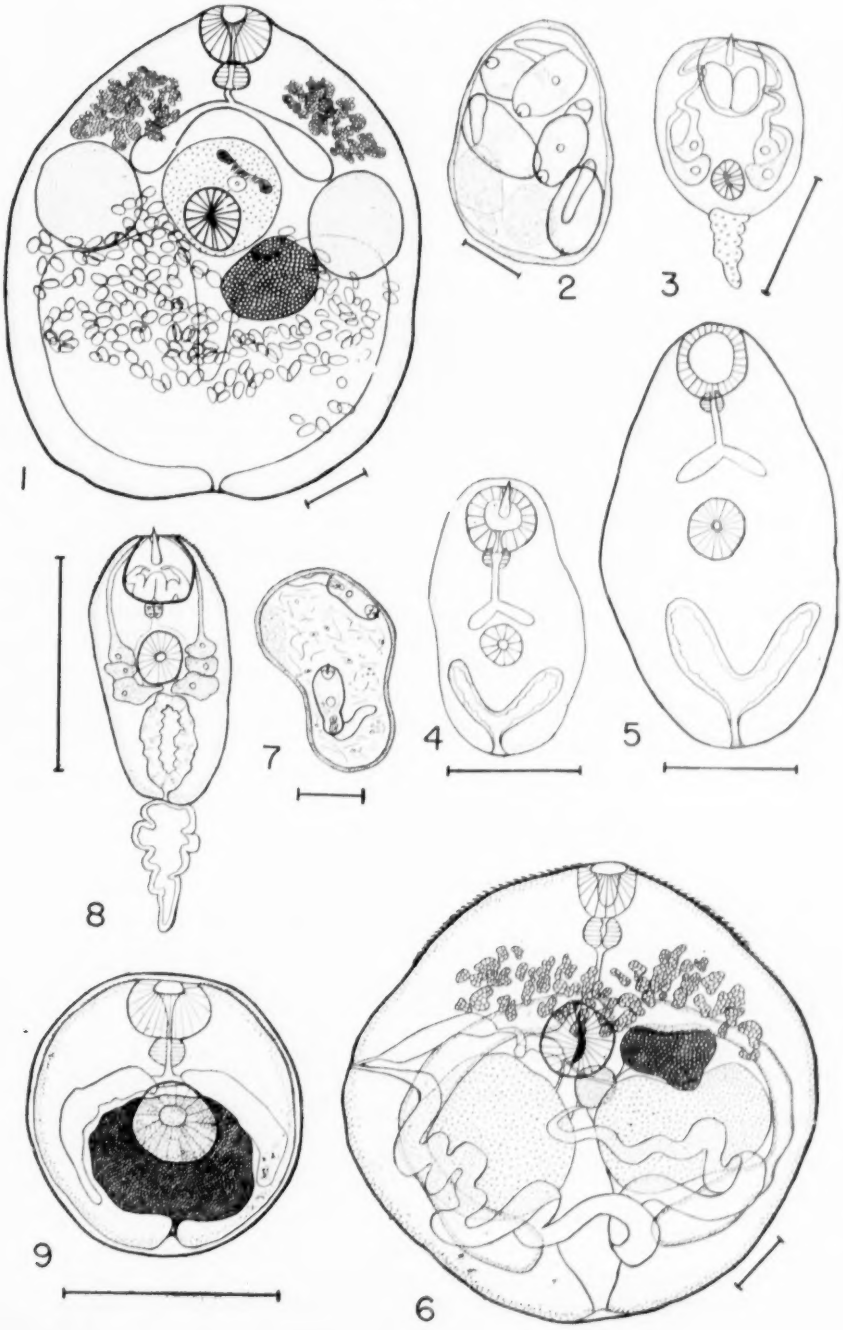
FIG. 6. Adult *Allassogonoporus vespertilionis* from the intestine of the hamster.

FIG. 7. Sporocyst of *Allassogonoporus vespertilionis* from *Fluminicola virens* showing 2 mature cercariae.

FIG. 8. Cercaria of *Allassogonoporus vespertilionis*.

FIG. 9. Encysted metacercaria of *Allassogonoporus vespertilionis*.

PLATE I



A NEW SPECIES OF BOTHRIOCEPHALID CESTODE, FROM THE FOX SQUIRREL (*SCIURUS NIGER* LINN.)

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and
University of Oklahoma Biological Station
University of Oklahoma

A specimen of *Sciurus niger*, collected near the Lake Texoma Biological Station, harbored an undescribed species of cestode belonging to the genus *Bothriocephalus*. To the authors' knowledge, this is the first reported occurrence of a member of this genus in a mammal. The specimen is sexually mature and the uterus in the posterior segments contains fully developed eggs. This unusual host relationship and certain distinct features of the worm justify in our opinion the recognition of a new species.

MATERIALS AND METHODS

Immediately after the cestode was removed from the stomach of the host, it was placed in water and drawings and measurements were made from the living specimen. It was not attached and is presumed to have moved into the stomach from the intestine after the host was killed. There was no food in the stomach of the host, and although there is the possibility that the worm was eaten with another animal harboring it, it is not likely that it would have remained intact under such conditions. Measurements were obtained by means of an ocular micrometer and tracings by microprojection. The specimen was fixed in F. A. A., stained with Ehrlich's acid hematoxylin, and all but 4 segments were mounted *in toto* in clarite. The segments not mounted *in toto* were sectioned for studies of internal structures.

Bothriocephalus sciuri n. sp.
(Figs. 1, 2, 3)

Length 94.0 mm. Mature segments 0.72 mm. wide by 0.27 mm. long; the oldest ones 0.87 mm. wide by 0.20 mm. long. Proglottid divided distally into 4 segments.

Scolex 0.97 mm. long, 0.27 mm. wide at terminal disc, 0.31 mm. wide in center and tapering to 0.07 mm. wide at posterior end, with 2 shallow grooves. Terminal disc bilobed and indented on the surficial surfaces, indentations connected by shallow groove (Fig. 1). Ootype immediately anterior to isthmus of ovary, and averaging 0.28 mm. in diameter. Vitellaria cortical and lateral, and continuous around lateral edge of proglottid and from segment to segment. Follicles numbering 800–1000 per segment and 27 microns in diameter.

Testes averaging 95 per segment, 58 microns in diameter, occupying lateral medullary position. Cirrus pouch near center of proglottid, on or near median axis; length 0.16 mm., width 0.12 mm. Cirrus organ tube-like and slightly anterior to center of segment. Vas deferens closely coiled and next to cirrus sac.

Uterus coiled in posterior segments and containing several eggs. Uterine pore ventral, nearly median and in anterior third of segment. Eggs operculate, averaging 60 by 29 microns. Vagina extending from ootype to external opening next to genital pore in cirrovaginal atrium. Seminal receptacle absent.

Longitudinal musculature in weak strands.

Host: Sciurus niger

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Organ: Stomach
Locality: Willis, Oklahoma
Type Specimen: U. S. Nat. Mus. Helm. Coll. No. 49484.

DISCUSSION

Only one bothriocephalid cestode is common in the vicinity of the University of Oklahoma Biological Station. This is *B. texomensis* (Self, 1954) which occurs in the Goldeye, *Hiodon alosoides*. Very distinct differences occur between the two, especially in the size and number of the vitellaria and testes, the form of the scolex, and the appearance of the musculature.

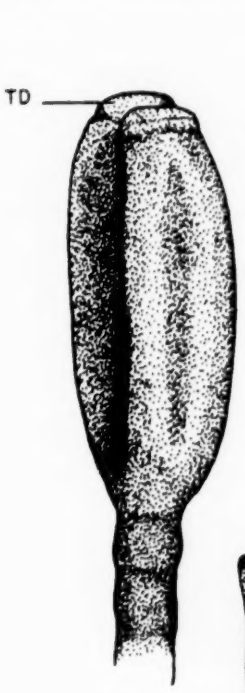

Fig. 1

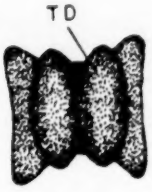

Fig. 2

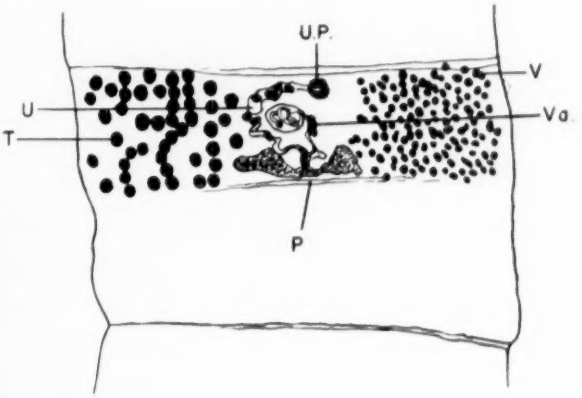

Fig. 3

EXPLANATION OF FIGURES

FIG. 1. Scolex showing one surficial groove and one lateral surface.
FIG. 2. End view of scolex showing lobed condition of terminal disc.
FIG. 3. Part of mature proglottid with one segment shown in detail.
Abbreviations: U. uterus; T. testis; P. partial segmentation of proglottid; Va. vagina; V. vitellaria; UP. uterine pore.

The number of testes averages about one-third more and the number of vitellaria about twice the number in *B. texomensis*. Also the scoleces are quite different. The scolex of this worm shows a pronounced notch and lobing of the terminal disc as opposed to the absence of lobing of the terminal disc in *B. texomensis*.

There is a resemblance of this tapeworm to *B. cuspidatus* (Cooper, 1917, 1918) but the number of testes is greater, the number of subsegments is 4 instead of 32;

the scolex is relatively much larger and the terminal disc shows a more pronounced lobing than in *B. cuspidatus*. The authors see little to be gained in establishing subspecies as has been done in certain cases (Wardle 1932), and establish the new species because of the distinct morphological characters mentioned and the unique host.

SUMMARY

Bothriocephalus sciuri n. sp. is reported from the fox squirrel (*Sciuris niger*). This is believed to be the first report of this genus of cestode from a mammal. Its closest relative seems to be *B. cuspidatus*, but certain distinct morphological features plus the unique host relationship justify the establishment of a new species.

LITERATURE CITED

COOPER, A. R. 1917 A morphological study of bothriocephalid cestodes from fishes. J. Parasit. **4**: 33–39.

———1918 North American pseudophyllidean cestodes from fishes. Ill. Biol. Monogr. **4**: 288–541.

SELF, J. T. 1954 Parasites of the goldeye *Hiodon alosoides* (Raf.), in Lake Texoma. J. Parasit. **40**: 386–389.

WARDLE, R. A. 1932 The Cestoda of Canadian Fishes II. The Hudson Bay drainage system. Contrib. Canad. Biol. and Fish. **7** (art. 30) Ser. A, Gen. No. 27: [377]–403.

THE QUANTITATIVE DETERMINATION OF GLYCOGEN IN SOME PARASITES OF AMPHIBIA*

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INTRODUCTION

Histological and histochemical techniques have been employed by a number of investigators in attempts to determine the distribution and amount of glycogen present in the tissues of various flatworms. Ortner-Schönbach (1913) reported glycogen in the muscle cells and parenchyma of *Haplometra cylindracea* from the lungs of *Rana temporaria* and from similar tissues in *Gorgodera cygnoides* and *Polystomum integerrimum* from the urinary bladder of *Rana esculenta*. Wilmoth and Goldfischer (1945), employing Best's carmine technique, Lugol's solution, and the Feulgen-Bauer reaction, identified glycogen granules in muscle tissue, eggs, and parenchyma of *Haematoloechus* sp. from *Rana pipiens*. Axmann (1947) found glycogen in the form of granules, fibers, and lumps in the oral and ventral suckers of *Haematoloechus*, *Gorgodera*, and *Gorgoderina*.

The glycogen content of a number of parasitic worms has been determined quantitatively, the majority of the forms being cestodes and nematodes; determinations have been made for only three trematodes. Flury and Leeb (1926) and Weinland and von Brand (1926) found 15% and 21% respectively, of the dry weight of *Fasciola hepatica* to be composed of glycogen. Bueding and Koletsky (1950), working with *Schistosoma mansoni*, reported a sex difference with respect to glycogen content; they found 13.6–29% of the dry weight of males to be glycogen but only 2.7% of the female composed of the same polysaccharide. Bair (1954)

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found that the glycogen content of *Haematoloechus medioplexus* varied from 0.50% to 1.37% of the wet weight of the parasites.

Various techniques have been devised in an attempt to demonstrate, quantitatively, the amount of glycogen present in the tissues of various flatworms (Whitacre, 1931; Good, Kramer, and Somogyi, 1933; Heatley, 1935; von Brand, 1936; Somogyi, 1945). In general, these methods follow a somewhat similar procedure; the reduction of the body tissues with strong alkali, precipitation of the glycogen by alcohol, followed by acid hydrolysis of the glycogen to glucose. The amount of glucose is then determined by titrating with sodium thiosulphate.

In the present work, a modified technique was used based on the procedure of Stiles, Peterson, and Fred (1926) for glucose determinations in bacteria. In order to test this technique against one utilizing the precipitation of glycogen by alcohol before acid hydrolysis, the method of Heatley (1935) was used. Two groups of frog lung flukes were treated in the same manner except that alcohol was added to the alkaline mixture resulting from the reduction with KOH of the tissues of one group, whereas the second group of flukes was treated without using alcohol. The glycogen content of the alcohol-treated group was 1.33 mg., that of the group of worms without alcohol, 1.27 mg. Since there appeared to be no significant difference between the two values obtained, the more rapid method of Stiles, Peterson, and Fred (1926) was employed.

MATERIALS AND METHODS

Worms were removed from the host, dehydrated over a boiling water-bath until they were a chalky-white, and then weighed. They were then placed in a large (200 by 25 mm.) test-tube together with 2 cc. of a 30% KOH solution; the tube was placed in a boiling water-bath and the parasite tissue allowed to digest for 30 minutes; the tube was shaken at intervals to insure thorough mixing of the tissue and the KOH. The tube was then removed from the water-bath and a few drops of phenolphthalein added together with enough 2*N* sulphuric acid to make the solution slightly acid. 3 cc. of 2*N* sulphuric acid were added to the tube which was replaced in the water-bath and the glycogen previously freed from the parasite tissue was hydrolyzed for two hours. The tube was then removed and cooled and the contents neutralized with 1*N* NaOH. This material was placed in a 10 cc. volumetric flask and brought up to the mark by adding distilled water. After mixing thoroughly, 5 cc. were withdrawn with a volumetric pipette; the sample, and the 5 cc. remaining in the flask, were placed in each of the two test-tubes. To each of two other tubes was added 5 cc. of distilled water; these served as "blanks" or controls.

A combined micro-reagent was prepared in the following way: 200 grams of anhydrous sodium carbonate were dissolved in 2000 cc. of warm distilled water. 25 grams of $CuSO_4$ and 37.5 grams of tartaric acid were first dissolved in 750 cc. of distilled water and then, with constant, vigorous stirring, were added slowly to the sodium carbonate solution. 50 grams of KI, 3.5 grams KIO_3, and 92 grams of potassium oxalate were dissolved in 1250 cc. distilled water and this mixture was added to the alkaline-copper solution which was allowed to cool, and then diluted up to 5 liters. This large amount was made in order to reduce the possibility of error present in making up small amounts.

5 cc. of the micro-reagent were added to each of the two tubes containing the test solution and to each of the two blanks. The tubes were capped with glass marbles to act as condensers and placed in a boiling water-bath for 15 minutes; at the end of this time, they were removed and cooled to room temperature. To each tube was then added 5 cc. of 1N H_2SO_4 and the mixture was titrated against 0.005N sodium thiosulphate to a starch end-point. The amount of glucose present was determined by comparing the titration difference between test solutions and blanks with a table of values previously established by conducting the test using standard glucose solutions. The glucose value was then multiplied by 0.927, the correction factor of Pflüger, to determine the amount of glycogen present.

DISCUSSION

Both trematodes and cestodes from frogs, *Rana pipiens*, *Rana clamitans*, and *Rana septentrionalis*, were used in an effort, first, to determine quantitatively, the amount of glycogen present and, second, to learn whether there is any correlation between the amount of glycogen and the habitat of the parasite within the host.

The results of the study are summarized in Table I:

TABLE I.—*Percentage of mgs. of glycogen to dry weight of some helminth parasites of frogs.*

Parasite	Dry wt. mg.	No. of worms	Glycogen mg.	Percent glycogen to dry wt.
Haematoloechus complexus	62	6	0.23	0.37%
Haematoloechus medioplexus	66	20	0.556	0.81
Haematoloechus medioplexus	42	6	0.389	0.92
Gorgodera amplicava	30	15	0.25	0.83
Gorgoderina attenuata	28	14	0.24	0.85
Clinostomum attenuatum metacercariae	63	12	1.04	1.66
Clinostomum attenuatum metacercariae	12	4	0.185	1.54
Crepidobothrium saphena	22	1	0.278	1.26
Crepidobothrium saphena	16	3	0.185	1.15

The percentage values of glycogen to dry weight listed in Table I indicate that the lung flukes, *H. complexus* and *H. medioplexus*, have significantly smaller amounts of glycogen than do the encysted *Clinostomum* metacercariae and the frog tapeworm, *Crepidobothrium saphena*. This is further substantiation of the observation of von Brand (1952) that "The parasites living in oxygen-poor habitats or in habitats with periodic oxygen deficiencies (stomach) regularly have a high glycogen content." Axmann (1947), on a purely morphological basis, found no clear-cut connection between the availability of oxygen in the various habitats and glycogen storage by the parasite occupying that habitat. She concluded, however, that the size of the parasite was a factor and that large flukes such as *Fasciola*, *Fascioloides*, and *Alassostoma* stored greater quantities of glycogen than did smaller worms in the same oxygen-poor habitat.

Glycogen content of the frog bladder flukes, *Gorgodera amplicava* and *Gorgoderina attenuata*, appears to be at variance with the generalization that parasites in oxygen-poor environments possess greater amounts of glycogen than do those inhabiting oxygen-rich habitats. On the basis of the relatively large amount of glycogen present in parasites such as *Crepidobothrium saphena* of the intestinal tract, a habitat deficient in oxygen, it might be expected that those forms found in the urinary bladder would also show a high glycogen content; this, however,

is not the case. Reference to Table I shows the glycogen content of lung and bladder flukes to be about equal. Gorgoderiid worms are small-bodied and thus, because of a relatively larger surface, may be capable of leading a more aerobic existence in an oxygen-deficient environment than would be possible in a larger-bodied fluke such as *Haematoloechus*.

One group of 4 *Clinostomum* metacercariae were removed from the abdominal muscles of *Rana pipiens* which had been starved in an aquarium for two months. The body of the host was thin and emaciated but the worms appeared to be in good condition and exhibited their usual rapid contractions when released from the cysts. Von Brand (1938) has noted that the larvae of *Eustrongylides ignotus* showed no diminution in glycogen content following starvation of the host, *Fundulus*, for a period of 65 days. Analysis of the two groups of *Clinostomum* metacercariae, the one group of four from a starved *Rana pipiens*, and another group of 12 from a *Rana pipiens*, in good condition, showed no significant difference in glycogen content. These results, as well as those of von Brand (1938) are in marked contrast to the observation of Reid (1942) on *Raillietina cesticillus* and of Reid (1944, 1945) on *Ascaridia galli* where starvation of the hosts for a period of 20–48 hours resulted in marked glycogen depletion of the parasites and actual loss of the worms from the intestinal tract.

CONCLUSIONS

Quantitative determinations of glycogen have been made for certain lung, tissue, and bladder flukes of frogs and for a cestode from a frog host. Lung flukes, in an oxygen-rich environment, possessed less glycogen than did the cestode from the intestine, a habitat poor in oxygen. Bladder flukes, from the relatively oxygen-deficient urinary bladder, showed a glycogen content closer to that of the lung forms rather than to the intestinal forms; this might be the result of a more aerobic existence made possible by greater body surface. Starvation of the host appeared to have little effect on glycogen content of encysted forms within the host.

LITERATURE CITED

AXMANN, M. C. 1947 Morphological studies on glycogen deposition in schistosomes and other flukes. J. Morphol. **80**: 321–334.

BAIR, T. D. 1954 Host-parasite glycogen relationships in *Haematoloechus medioplexus*. J. Parasit. **40** (5–Sect. 2): 43–44.

VON BRAND, TH. 1936 A rapid working micro-modification of Pflüger's glycogen method. Skandin. Arch. f. Physiol. **75**: 195–198.

———1938 Physiological observations on a larval *Eustrongylides* (Nematoda). J. Parasit. **24**: 445–451.

———1952 Chemical Physiology of Endoparasitic Animals. Academic Press, N. Y. 339 pp.

BUEDING, E. AND KOLETSKY, S. 1950 Content and distribution of glycogen in *Schistosoma mansoni*. Proc. Soc. Exp. Biol. and Med. **73**: 594–596.

FLURY, F. AND LEEB, F. 1926 Zur Chemie und Toxikologie der Distomen (Leberegel). Klin. Wochschr. **5**: 2054–2055.

GOOD, C. A., KRAMER, H. AND SOMOGYI, M. 1933 The determination of glycogen. J. Biol. Chem. **100**: 485–491.

HEATLEY, N. G. 1935 The distribution of glycogen in the regions of the amphibian gastrula; with a method for the micro determination of glycogen. Biochem. Jour. **29**: 2568–2572.

ORTNER-SCHÖNBACH, P. 1913 Zur Morphologie des Glykogens bei Trematoden und Cestoden. Archiv Zellforsch. **11**: 413–449.

REID, W. M. 1942 Certain nutritional requirements of the fowl cestode *Raillietina cesticillus* (Molin) as demonstrated by short periods of starvation of the host. J. Parasit. **28**: 319–340.

———1944 The effects of host starvation on worm elimination and glycogen depletion with the nematode *Ascaridia galli*. J. Parasit. **30** (Aug. Suppl.) : 12.
———1945 Comparison between in vitro and in vivo glycogen utilization in the fowl nematode *Ascaridia galli*. J. Parasit. **31**: 406–410.
SOMOGYI, M. 1945 A new reagent for the determination of sugars. J. Biol. Chem. **160**: 61–68.
STILES, H. R., PETERSON, W. H. AND FRED, E. B. 1926 A rapid method for the determination of sugar in bacterial cultures. J. Bact. **12**: 427–439.
WEINLAND, E. AND VON BRAND, TH. 1926 Beobachtungen an *Fasciola hepatica* (Stoffwechsel und Lebensweise). Zeitschr. vergleich. Physiol. **4**: 212–285.
WHITACRE, H. M. 1931 Application of a micro-chemical method to the quantitative estimation of glycogen in bacteria. Master's Thesis, Ohio State Univ., 1931, 35 pp.
WILMOTH, J. H. AND GOLDFISCHER, RHODA 1945 On the distribution of glycogen in the trematode, *Ostiolum* sp. J. Parasit. **31** (Aug. Suppl.) : 22.

THE MORPHOLOGY OF *GIGANTOBILHARZIA HUTTONI* (LEIGH, 1953) AN AVIAN SCHISTOSOME WITH MARINE DERMATITIS-PRODUCING LARVAE

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Cercaria huttoni Leigh, 1953 was the third of four marine schistosome cercariae that have been reported as producing a dermatitis in man experimentally or as a result of natural exposure.

Prior to 1950, when Penner reported that *Cercaria littorinalinae* from the marine snail *Littorina planaxis* produced a dermatitis experimentally, cercarial dermatitis was regarded as a fresh water involvement. Stunkard and Hinchliffe (1952) reported that a dermatitis of marine origin in Narragansett Bay, Rhode Island, was caused by *Cercaria variglandis* Miller and Northup, 1926 from the marine snail, *Nassa obsoleta*. Penner (1953a) reported the recovery of a fourth marine dermatitis-producing cercaria in *Batillaria minima* off the west coast of Florida. Chu (1952) tentatively identified a dermatitis-producing cercaria from *Littorina pintado* in Hawaii as *C. littorinalinae* Penner, 1950, but later (Chu and Cutress, 1954) reported it to develop into *Austrobilharzia variglandis* (Miller and Northup, 1926).

Interest in schistosome cercariae in the United States dates back to a report by Cort (1928) that these larvae were responsible for "swimmer's itch" in fresh water lakes. In 1950 he recorded 18 species of non-human schistosome cercariae that have been implicated in the production of dermatitis and gave a complete summary of the work that had been done on schistosome dermatitis throughout the world.

Avian schistosomes of the subfamily BILHARZIELLINAE (family SCHISTOSOMATIDAE), to which the adult of *C. huttoni* belongs, are long, slender, fragile worms which usually inhabit the intestinal veins of their hosts. Due to the difficulties involved in finding and extricating them intact from these minute vessels, satisfactory specimens are often not obtained and there is confusion regarding the morphology and taxonomy of some species within the subfamily, due to inadequate material. Life history studies prove difficult for the same reason.

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Oiso (1927) reported the first avian schistosome life history for *Bilharziella yokogawai*. McLeod (1937) transferred this species to the genus *Pseudobilharziella*. The second life history was that of *Bilharziella polonica* reported by Szidat (1929). Brumpt (1931) discovered that *Cercaria ocellata* LaValette, 1855 developed into *Trichobilharzia kossarewi* Skrjabin and Zakharow, 1920. Brackett (1942) recovered the adult of *Cercaria gyrauli* Brackett, 1940, from blackbirds and placed it in the genus *Gigantobilharzia*. He also described four new species of schistosomes in the genus *Pseudobilharziella*, and *Gigantobilharzia lawayi*, from gulls. McLeod and Little (1942) determined that *Cercaria physellae* Talbot, 1936 was the larval stage of *Pseudobilharziella querquedulae* McLeod, 1937.

McMullen and Beaver (1945) gave a complete account of three life histories in the genus *Trichobilharzia* and reduced the genus *Pseudobilharziella* Ejsmont, 1929 to synonomy with *Trichobilharzia*, incorporating species from the former genus into the latter. Stunkard and Hinchliffe (1952) recovered experimentally produced adults of *Cercaria variglandis* Miller and Northup, 1926 and found them to be identical with specimens of *Microbilharzia chapini* Price, 1929. Penner (1953b), on the basis of material recovered from the red-breasted merganser and several experimental hosts, reduced the genus *Microbilharzia* Price, 1929 to synonomy with *Austrobilharzia* Johnson, 1917 and transferred the adult of *Cercaria variglandis* to the latter genus. Najim (1952) worked out the life history of *Gigantobilharzia huronensis* Najim, 1950 from gold finches and cardinals. Penner (1953a) infected pigeons with a marine dermatitis-producing cercaria from *Batillaria minima* and the adults recovered from pigeons and naturally infected royal terns were identified as *Ornithobilharzia canaliculata* (Rudolphi, 1819). Penner (1953c) also reported that *Cercaria littorinalinae* Penner, 1950 developed in several avian hosts into adults which belong in the genus *Austrobilharzia*.

A study of the morphology of the adults of *Cercaria huttoni* indicates that they belong in the genus *Gigantobilharzia* Odhner, 1910; therefore, the correct name for this schistosome is *Gigantobilharzia huttoni* (Leigh, 1953).

METHODS

Pigeons, young chicks and parakeets were tried as experimental hosts for *G. huttoni*. Only the parakeets were positive on examination. In all cases birds were exposed after clipping all breast feathers down to the bare skin. Since the cercariae lie in contact with the surface film and would be easily picked up on the feathers, and perhaps fail to penetrate, the exposed skin was repeatedly lowered to the surface film to insure contact with many cercariae. A microscopic check of the water indicated that cercariae made a successful transfer to the skin. The birds were held in contact with the surface film for thirty minutes, then confined for 15 to 30 minutes more with the lower parts of their bodies immersed in sea water. No attempt was made to infect birds by drinking water, since sea water is the normal environment of the cercariae.

Location and removal of the adult schistosomes involve considerable frustration. Other investigators have mentioned the difficulties of removing intact the fragile slender adults from the minute intestinal veins in which they live. Adult *G. huttoni* were taken only from the small veins in the intestinal wall, sometimes apparently in the submucosa. They were most easily removed by scraping off the mucosa.

The worms could be seen through the outer wall of the intestine by the aid of a dissecting microscope only when the intestinal ceca were filled with a brown pigment. Usually it was necessary to slit the intestine, remove the contents, and flatten the organ between two microscopic slides to locate the worms. The males usually lacked the brown pigment in the intestine and could be discovered only by removing the mucosa. The fragile character of the worms and the narrow and sometimes tortuous vessels in which they are confined make removal difficult.

On removal, the adults were transferred to saline and fixed in hot AFA solution or Gilson's fixative at 60° C. In most cases the worms relaxed reasonably well but did not straighten out. Mayer's carmalum and alum cochineal were used as stains. Ten complete females, two intact males, numerous fragments and specimens nearly complete were available for measurements and morphological study. The more slender and fragile character of the male is reflected in the small number that were removed intact. Except for length measurements on the male, the material available for study was regarded as adequate.

THE GENUS *GIGANTOBILHARZIA* ODHNER, 1910

Price (1929) divided the family SCHISTOSOMIDAE into two subfamilies, SCHISTOSOMINAE Stiles and Hassall, 1898 and BILHARZIELLINAE, a new subfamily. The latter subfamily was characterized as follows: "Male and female similar in form, either flattened or threadlike. Suckers present or absent. Gynaecophoric canal absent or imperfectly formed. Paired intestinal ceca short; common cecum long, with or without lateral dendritic branches. Testes numerous and situated along the course of a common cecum. Uterus short and containing a single egg." In the subfamily the genera *Bilharziella* Odhner and *Dendritobilharzia* Skrjabin and Zakharow, 1920 have broad flattened bodies. With the reduction of *Pseudobilharziella* Ejsmont, 1929 to synonomy by McMullen and Beaver (1945), the genera *Trichobilharzia* Skrjabin and Zakharow, 1920 and *Gigantobilharzia* Odhner, 1910, with threadlike bodies, complete the subfamily.

Odhner's (1910) characterization of the genus *Gigantobilharzia* was based on *G. acotylea* and is now quite inadequate. Brackett (1942) suggested that the species now included in the genus may not be cogeneric. Until the group can be more carefully studied and the generic diagnosis revised, it would appear that consideration should be given to the following characters. Body slender, threadlike, and similar in both sexes. Oral sucker present or absent, acetabulum lacking. Digestive system bifurcating near anterior end and reuniting anterior to or in the vicinity of gynaecophoric canal in the male or seminal receptacle of female to form a common cecum extending to the posterior end. Gynaecophoric canal sometimes absent; when present, it is short and located in approximately the anterior fifth of the body. Eggs round to oval and without spines or possessing only a rudimentary terminal spine. Uterus containing one to several eggs, female genital pore opening at or near anterior end.

McMullen and Beaver (1945) doubted the accuracy of reports that the female genital pore in the genus *Gigantobilharzia* opened at the oral sucker or, in its absence, the equivalent area. They suggested a re-examination of this relationship. This anterior location was clearly seen in several female specimens of *G. huttoni* and is undoubtedly a valid character for the genus.

GIGANTOBILHARZIA HUTTONI (LEIGH, 1953)
(Figs. 1 to 7)

Specific description (measurements in millimeters): *Gigantobilharzia:* Filamentous, cylindrical worms. Sexes of similar form, males shorter and more slender than females. Posterior extremities of both sexes lacking any pronounced enlargement or lobelike projections. Oral and ventral suckers absent. Mouth subterminal. Entire cuticula of male and female marked by striations.

Male: About 4.72 long; 0.035 to 0.042 wide in region of gynaecophoric canal, 0.022 to 0.025 wide anterior to and 0.022 to 0.032 posterior to gynaecophoric canal. Esophagus long, bifurcating a little distance anterior to seminal vesicle; intestinal ceca uniting in posterior third of gynaecophoric canal and well anterior to testes. Brown pigment in intestine scarce or entirely absent in male. Gynaecophoric canal short, beginning 0.960 to 1.12 from anterior end and extending for a distance of 0.235 to 0.330; edges marked by conspicuous transverse cuticular folds. Testes spherical to ovoid, about 0.032 long by 0.024 wide, approximately 60 in number arranged in longitudinal series. Testicular field beginning about 0.200 posterior to gynaecophoric canal and extending almost to end of body. Vas deferens not traced, but seminal vesicle beginning 0.400 to 0.635 from the anterior end, describing a tortuous backward course, passing through a cluster of prostate cells. Cirrus sac not seen but probably present; ejaculatory duct leading to prominent spinous protrusible cirrus medially situated in anterior portion of gynaecophoric canal.

Female: Larger than male, 5.26 to 7.22 long; 0.032 to 0.042 wide at level of ovary, 0.029 to 0.035 wide anterior to and 0.038 to 0.048 posterior to ovary. Esophagus bifurcating anterior to oötype, about 0.276 to 0.386 from anterior end, ceca uniting immediately posterior to seminal receptacle, intestine containing considerable brown pigment. Ovary irregular in outline, beginning about 0.490 to 0.648 from anterior end, and 0.230 to 0.386 long; oviduct narrow, dilating into a convoluted seminal receptacle, turning and proceeding anteriorly as a slender duct to the oötype about 0.090 to 0.170 anterior to the ovary. Uterus relatively long. Genital pore near and posterior to mouth opening. Vitelline follicles beginning short distance posterior to seminal receptacle, packing body to posterior end. Mature embryonated eggs from feces oval, 0.090 to 0.099 long by 0.061 to 0.067 wide, lacking spine but occasionally rudimentary terminal spine present. Eggs deposited in intestinal villi and hatching in saline on release from intestinal mucosa.

Experimental Host: Parakeet. Natural host unknown.

Location: Small veins of the small intestine.

Locality: Snail host, *Haminoea antillarum guadalupensis*, from Virginia Key, Florida.

Cotypes: Helminth. Coll. U. S. Nat. Museum No. 37473 and author's collection.

DISCUSSION

Seven species have been described for the genus *Gigantobilharzia*. *G. sturniae* Tanabe, 1948 was too poorly described to permit adequate comparisons. *G. monocotylea* Szidat, 1930 from gulls and *G. egreta* Lal, 1937 from the cattle egret were described as lacking a gynaecophoric canal which *G. huttoni* possesses. *G. gyrauli* (Brackett, 1940) from blackbirds has well developed oral suckers in both sexes; *G. huttoni* lacks them in both sexes as do the remaining species in the genus. *G. acotylea* Odhner, 1910, the type species, has posterior lobe-like projections and is 140 to 165 mm. long compared with less than 10 mm. for *G. huttoni*.

G. huttoni most closely resembles *G. lawayi* Brackett, 1942 from gulls and *G. huronensis* Najim, 1950 from the goldfinch and cardinal but is easily distinguished from both. *G. lawayi* males are wider than females and are three to four times as wide as *G. huttoni* males. *G. lawayi* males possess a gynaecophoric canal 2.25 mm. in length as compared with a maximum length of 0.330 mm. in *G. huttoni*. The testes in the former species number over 500, more than five times as numerous as in *G. huttoni*.

G. huronensis females are also longer than males, but the 16 to 29 mm. length for females greatly exceeds the 7.22 mm. maximum for *G. huttoni* females. The possession by the male *G. huronensis* of a gynaecophoric canal 1.084 mm. in length,

over 300 testes and intestinal ceca that unite far anterior to the gynaecophoric canal additionally separate the males. *G. huronensis* females have more rounded eggs and as many as seven in the uterus at one time. No more than one has been seen in the uterus of *G. huttoni*.

All six parakeets exposed to the cercariae of *G. huttoni* became infected. Only a few adults were recovered from the first two birds examined at 30 and 47 days following exposure. No eggs were seen in the mucosa. A third bird appeared ill and was killed on the 53rd day. Almost the entire mucosa of the small intestine was loaded with eggs, one to three per villus, but only about 15 worms were recovered from the intestine. The fourth bird was killed on the 77th day and was estimated to have 40 to 50 worms. Most were in the middle third of the small intestine and males were more numerous than females. Lacking the cecal pigment, the males could be seen only by gradually shredding the intestinal wall. Numerous active miracidia hatched within a few minutes after removal of the split intestine to saline solution.

After 124 days parakeet No. 5 became ill and refused to eat. Its fecal matter was pasty and accumulated around the anus. Examination of feces revealed numerous eggs in various stages of development, some hatching in saline solution. Tissues of the entire small intestine were massively infected with eggs in all stages of development including those which released miracidia. Most villi were greatly enlarged and pendulous from the 15–20 eggs which each contained. The mucosa appeared to have undergone considerable sloughing before the bird was killed. This was further indicated by the numerous eggs passed in feces in early stages of development. In contrast, parakeet No. 6, apparently a less heavily infected bird, passed only eggs containing mature miracidia. The intestinal wall of bird No. 5 contained numerous adults, mostly females, which were concentrated in the posterior two-thirds of the small intestine. A few eggs and an occasional adult worm were noted in the anterior part of the large intestine.

Apparently eggs are deposited in or near the villi, since they were not seen elsewhere. Since only an occasional egg could be found in the liver of the most heavily infected bird, it seems likely that most eggs are retained within the intestinal wall. Parakeet No. 6 was still passing eggs with active miracidia 10 months after infection.

No attempt has been made so far to study migration and morphology of the early developmental stages in the avian host. The only immature forms seen were three taken from the liver of a parakeet with a 47 day old infection.

The natural host for this parasite has not been ascertained. Ruddy turnstones, black skimmers, laughing gulls, black-bellied plovers, dowitchers, and least terns were examined from the area where snails were infected with *C. huttoni*. Failure to find *G. huttoni* in these birds is not final, since the examination occurred earlier than the recovery from parakeets and it is possible that the worms were overlooked. Brackett's (1942) report of *G. lawayi* from herring and ring-billed gulls suggests that further attention be given to the gulls of the Miami area.

One correction needs to be made in Leigh's (1953) description of *Cercaria huttoni*, in which three pairs of penetration glands were reported in the emerged cercaria. Nuclei of the gland cells did not show in emerged cercariae, and the overlapping of the glands obscured the outlines of two pairs of glands that were then interpreted as lobes of the three pairs reported. Recently a phase microscope re-

vealed that cercariae dissected from the snail host have 6 pairs of gland cells. One small anterior pair empties its contents before emergence and does not appear in emerged cercariae. Emerged cercariae possess two sets of glands, an anterior set consisting of two pairs with finely granular contents and a posterior set consisting of three pairs of postacetabular glands with coarsely granular contents. Gland pairs 1, 2, and 4 are ventrally located; the third and fifth pairs are dorsal in location (Fig. 6). The ducts of the escape glands run lateral to the other ducts into the anterior organ, then cross these ducts ventrally to assume a medial position. The penetration ducts open at the bases of sharply pointed penetration spines.

Leigh (1953) discussed the role of *C. huttoni* as a dermatitis-producer and stated that the restricted distribution of the snail host, *Haminoea antillarum guadalupensis*, would probably preclude the possibility of *C. huttoni's* playing any important part in the marine dermatitis referred to in Florida as seabather's eruption. This involvement, associated with what the layman of this area calls "sea lice," occurs along the southeast coast of Florida from March to August with most cases from May to July. Hutton (1952) reported that schistosome cercariae were the probable cause of this dermatitis, but this opinion was based only on the finding of the cercaria later described as *C. huttoni*. Since Leigh (1953) reported a dermatitis-producing schistosome cercaria from *Nassarius vibex* in the Miami area, and Penner (1953a) reported another from *Batillaria minima* from the Gulf coast of Florida, it seems possible that schistosome cercariae other than *C. huttoni*, might be the cause of seabather's eruption, for these snails are numerous along the beaches of South Florida. However, the striking localization of seabather's eruption in the areas of the body covered by the bathing suit as compared with the more general distribution of the dermatitis in all known cases of fresh water and marine cercarial dermatitis suggests caution in associating the Florida eruption with schistosome cercariae at this time. Sams' (1949) histological examination of lesions excised from patients with seabather's eruption showed no foreign bodies or parasites, although none of the lesions were taken earlier than 24 hours, and it is possible that the causative agent would be disintegrated within that period.

ABSTRACT-SUMMARY

Pigeons, young chicks and parakeets were exposed to *Cercaria huttoni* Leigh, 1953, a dermatitis producing cercaria from the marine snail *Haminoea antillarum guadalupensis* collected at Virginia Key, Florida. Parakeets serve as adequate experimental hosts; pigeons and chicks were negative on examination. This schistosome species belongs to the genus *Gigantobilharzia* and the adult is confined to minute veins in the wall of the intestine. Oval unspined eggs passed in the feces hatch normally. The natural host of this parasite is unknown.

LITERATURE CITED

BRACKETT, S. 1942 Five new species of avian schistosomes from Wisconsin and Michigan with the life cycle of *Gigantobilharzia gyrauli* (Brackett, 1940). J. Parasit. **28**: 25–42.

BRUMPT, E. 1931 *Cercaria ocellata*, déterminant la dermatite des nageurs, provient d'une bilharzie des canards. Compt. Rendu Acad. Sc. Paris **193**: 612–614.

CHU, GEORGE W. T. C. 1952 First report of presence of dermatitis-producing marine larval schistosome in Hawaii. Science **115**: 151–153.

——— AND CUTRESS, C. E. 1954 *Austrobilharzia variglandis* (Miller and Northup, 1926) Penner, 1953, (Trematoda: Schistosomatidae) in Hawaii with notes on its biology. J. Parasit. **40**: 515–524.

CORT, W. W. 1928 Schistosome dermatitis in the United States (Michigan). J. Am. Med. Ass. **90**: 1027–1029.

———1950 Studies on schistosome dermatitis. XI. Status of knowledge after more than twenty years. Am. J. Hyg. **52**: 251–307.

HUTTON, R. F. 1952 Schistosome cercariae as the probable cause of seabather's eruption. Bull. Mar. Sci. Gulf and Caribbean **2**: 346–359.

LAL, M. B. 1937 Studies on the trematode parasites of birds. Part II. Morphology and systematic position of some new blood flukes of the family Schistosomatidae. Proc. Ind. Acad. Sc. **6** (Sect. B): 274–283.

LEIGH, W. H. 1953 *Cercaria huttoni*, sp. nov., a dermatitis-producing schistosome larva from the marine snail, *Haminoea antillarum guadalupensis* Sowerby. J. Parasit. **39**: 625–629.

MCLEOD, J. A. 1937 Two new schistosomid trematodes from water birds. J. Parasit. **23**: 456–466.

——— and LITTLE, G. E. 1942 Continued studies on cercarial dermatitis and the trematode family Schistosomatidae in Manitoba. Part I. Canad. J. Research **20** (Sect. D): 170–181.

MCMULLEN, D. B. AND BEAVER, P. S. 1945 Studies on schistosome dermatitis IX. The life cycle of three dermatitis-producing schistosomes from birds and a discussion of the subfamily Bilharziellinae (Trematoda: Schistosomatidae). Am. J. Hyg. **42**: 128–154.

MILLER, H. M. AND NORTHUP, F. E. 1926 The seasonal infestation of *Nassa obsoleta* (Say) with larval trematodes. Biol. Bull. **50**: 490–508.

NAJIM, A. T. 1952 Life history of *Gigantobilharzia huronensis* Najim, 1950, a dermatitis-producing bird blood-fluke (Trematoda-Schistosomatidae). Dissertation—99 pp. Univ. of Mich.

ODHNER, T. 1910 *Gigantobilharzia acotylea* n.g., n. sp., ein mit den Bilharzien verwandter Blutparasit von enormer Länge. Zool. Anz. **35**: 380–385.

OISO, T. 1927 On a new species of avian *Schistosoma* developing in the portal vein of the duck, and investigation of its life-history. [Japanese text] Taiwan Igakkwai Zasshi, Taihoku pp. 848-865 (English abstract, pp. 5–7).

PENNER, L. R. 1950 *Cercaria littorinalinae* sp. nov., a dermatitis-producing schistosome larva from the marine snail, *Littorina planaxis* Philippi. J. Parasit. **36**: 466–472.

———1953a The biology of a marine dermatitis-producing schistosome cercaria from *Batillaria minima* (Gmelin). J. Parasit. **39** (No. 4–Sect. 2): 19–20.

———1953b The red-breasted merganser as a natural avian host of the causative agent of clam diggers' itch. J. Parasit. **39** (No. 4–Sect. 2): 20.

———1953c Experimental infections of avian hosts with *Cercaria littorinalinae* Penner, 1950. J. Parasit. **39** (No. 4–Sect. 2): 20.

PRICE, E. W. 1929 A synopsis of the trematode family Schistosomidae with descriptions of new genera and species. Proc. U. S. Nat. Mus. **75** (Art. 18): 1–39.

SAMS, W. M. 1949 Seabather's eruption. Arch. Dermat. Syphilol. **60**: 227-235.

STUNKARD, H. W. AND HINCHLIFFE, M. C. 1952 The morphology and life history of *Microbilharzia variglandis* (Miller and Northup, 1926) Stunkard and Hinchliffe, 1951, avian blood-flukes whose larvae cause "swimmer's itch" of ocean beaches. J. Parasit. **38**: 248–265.

SZIDAT, L. 1929 Die Parasiten des Hausgeflügels. 3. *Bilharziella polonika* Kow., ein im Blut schmarotzender Trematode unserer Enten, seine Entwicklung und Uebertragung. Arch. Geflügelk. **3**: 78–87.

———1930 *Gigantobilharzia monocotylea* n. sp. ein neuer Blutparasit aus ostpreussischen Wasservögeln. Ztschr. Parasitenk. **2**: 583-588.

TANABE, H. 1951 *Cercaria segmentinae* Tanabe, 1948, a homonym of *Cercaria sturniae*. J. Parasit. **37**: 321–322.

EXPLANATION OF PLATE

(Figs. 1–5 drawn with the aid of a camera lucida)

FIG. 1. Anterior end of adult female, 77 days old, showing reproductive organs, genital pore and digestive system.

FIGS. 2 and 3. Anterior end of adult male, 77 days old, showing reproductive organs, gynaecophoric canal (stippled area) and digestive system. Fig. 3 is a direct continuation of Fig. 2. Remnants of cercarial eye-spots shown in Fig. 2.

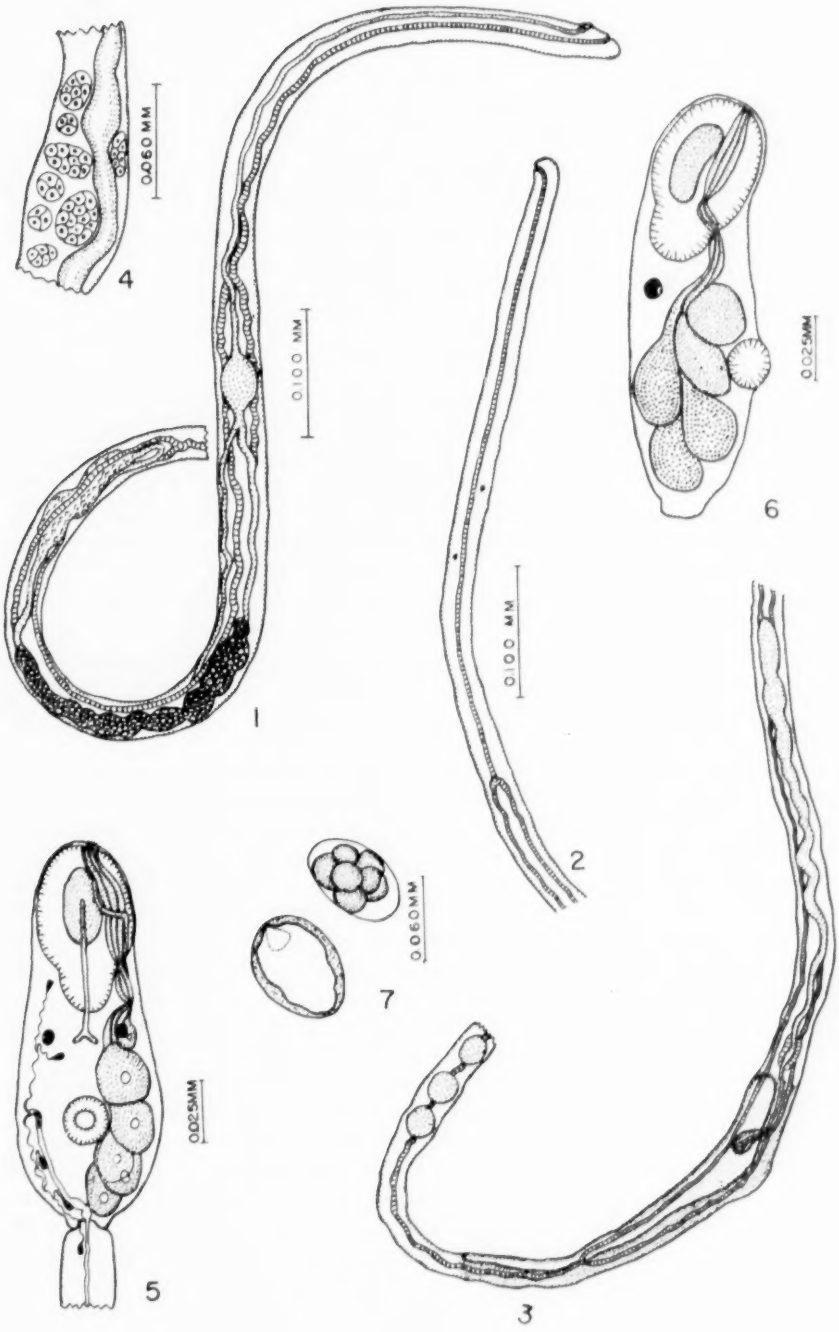
FIG. 4. Portion of posterior half of female to show disposition of vitellaria.

FIG. 5. Body of cercaria dissected from snail, showing number and position of penetration glands and flame cells.

FIG. 6. Body of emerged cercaria from lateral view to show positions of penetration glands. Drawn from living cercariae.

FIG. 7. Eggs, one in early cleavage, the other containing miracidium. Drawn from photomicrographs.

PLATE I



HYMENOLEPIS VIRILIS N. SP., A CESTODE FROM THE SHREW *SOREX TROWBRIDGEI* IN CALIFORNIA

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About 50 cestodes were recovered from the small intestine of two shrews collected August 22, 1954, two miles west of Bridgeville, Humboldt County, California. The cestodes were fixed in Bouin's fluid and stained with a very dilute aqueous solution of Ehrlich's haematoxylin. These specimens cannot be identified as any of the known species and they are therefore described as new. The following diagnosis of *Hymenolepis virilis* n. sp. is based on the study of 25 specimens.

Hymenolepis virilis n. sp.

Diagnosis: Strobila total length 1.0–2.1 mm. in mature specimens; greatest width 72–136 microns. Late mature and gravid proglottids longer than wide. Scolex with well developed rostellum bearing 10 hooks. Scolex diameter 108–180 microns, average 149 microns; sucker diameter 40–52 by 52–74 microns, average 47 by 63 microns; rostellar sac diameter 52–80 by 54–80 microns, average 63 by 66 microns; rostellum diameter 38–54 microns, average 46 microns; hook length 28–32 microns. Genital pores unilateral, situated in anterior third of lateral margin of segment. Testes arranged triangularly, diameter 24–40 microns, average 31 microns. Cirrus prominent and spinose; cirrus-pouch 50–90 microns long, average 67 microns, by 24–30 microns wide, average 26 microns; cirrus spines 12–16 microns long. Vagina with small seminal receptacle. Ovary bipartite, situated in anterior half of proglottid. Gravid uterus saccular nearly filling proglottid. Eggs ovoid, diameter 18–20 by 26–32 microns, average 19 by 28 microns.

Type host: Sorex trowbridgei.

Habitat: Intestine.

Type locality: Two mi. W. Bridgeville, Humboldt Co., California.

Type Specimen: U. S. Natl. Mus. Helm. Coll. No. 37442.

DESCRIPTION

The scolex (Fig. c) bears four suckers and a well developed rostellum crowned with ten hooks. The shape of the hooks (Fig. b) is sufficiently distinctive to separate this species from all others described from shrews. Posterior to the scolex there is a distinct neck, which leads to the immature region of the strobila. Immature proglottids are about twice as wide as long; the testes and the primordia of the cirrus-pouch are the only parts of the reproductive systems observed in the immature region (Fig. a). Mature proglottids are almost as long as wide. The cirrus is usually extruded and the female gonads are fully developed. In late mature proglottids, the testes decrease in size and are not easily observed. Gravid proglottids are longer than wide. The genital pores are situated in the anterior third of the lateral margin of the segment. The excretory system consists of two pairs of longitudinal excretory ducts (Fig. e); no cross-connections were observed.

Male reproductive system: The testes, as a rule, form a triangle; there are very few exceptions to this arrangement. In late mature proglottids, one may observe the vasa efferentia (Fig. e) leading from the testes to the narrow vas deferens which expands into a prominent external seminal vesicle and then narrows considerably before entering the cirrus-pouch, containing a well developed internal seminal vesicle. The cirrus-pouch is thin-walled and usually somewhat bent so

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that accurate length measurements are not always obtainable. The cirrus is of interest because it is very large in relation to the size of the proglottid; it is armed with relatively few, long spines (Fig. e).

Female reproductive system: The ovary consists of two or three large lobes and is situated in the anterior half of the proglottid. Posterior to it is the nearly spherical vitelline gland. The vagina expands into a small seminal receptacle dorsal to the ovary and proceeds as a straight and narrow tube toward the genital atrium which it enters either ventral to or just posterior to the cirrus. In late mature proglottids, a funnel-shaped enlargement of the distal portion of the vagina may be observed as well as a widening throughout its course. The uterus is anterior and dorsal to the ovary in early mature segments; in more posterior segments, it gradually enlarges and eventually fills the gravid proglottids (Fig. d). Eggs are thin-shelled and ovoid.

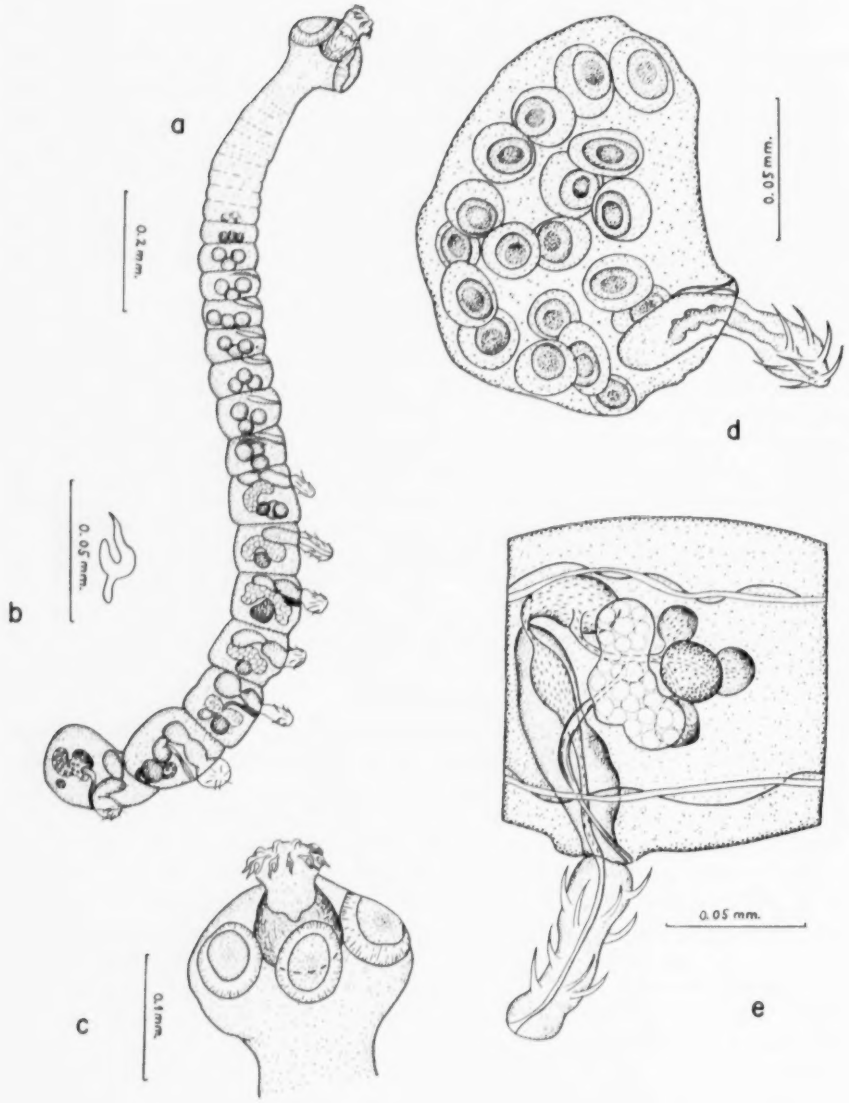
DISCUSSION–SUMMARY

Hymenolepis virilis n. sp. differs from all other hymenolepidids described from shrews in the shape of the rostellar hooks. Moreover, *H. virilis* has a very conspicuous cirrus and cirrus armature which has not been observed in any other known species. *H. virilis* resembles *H. lincola* Oswald, 1951, in number and size of hooks, and in position of testes. It differs from the latter species in shape of hooks, size and armature of cirrus, and in the size of the strobila and of various internal structures. The other hymenolepidid species from shrews differ markedly and can not be confused with *H. virilis*. No variability in hook number and shape occurred in the specimens of *H. virilis* studied. Slight variability was observed in position of testes, and considerable variability in size of the mature strobila and number of proglottids. The shape of mature and gravid proglottids was fairly constant in all specimens studied.

REFERENCE

OSWALD, V. H. 1951 Three new hymenolepidid cestodes from the smoky shrew, *Sorex fumeus* Miller. J. Parasit. **37**: 573–576.

PLATE I



EXPLANATION OF PLATE

All drawings made with the aid of a camera lucida.

Figs. a–e. *Hymenolepis virilis* n. sp. a, Whole worm; terminal gravid segment absent in this specimen; b, rostellar hook; c, scolex; d, gravid proglottid; e, late mature proglottid.

EFFECTS OF VARIATIONS IN RESERVOIR LEVELS, RAINFALL, AND TEMPERATURE ON ANOPHELINE DENSITIES IN A COASTAL PLAINS AREA IN SOUTH CAROLINA

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Communicable Disease Center, Public Health Service,
U. S. Department of Health, Education, and Welfare, Atlanta, Georgia;
The South Carolina State Board of Health, Columbia, South Carolina;
and the CDC Field Station,* Manning, South Carolina

During the course of malaria studies conducted for several years in the environs of the Santee Reservoir in east-central South Carolina, observations were made on abundance of anopheline mosquitoes. This report, which is based upon data obtained during the 5-year period 1947–1951, is concerned with the relations of densities of *Anopheles quadrimaculatus* and *A. crucians* to rainfall, temperature, and water level of the reservoir.

METHODS

Indices of mosquito populations were obtained by diurnal counts of adult mosquitoes in occupied mule stables. Counts were made once each week in 17 stations which were used throughout the study.

Reservoir levels were obtained at 7-day intervals. Rainfall and temperature data were secured from the records of the U. S. Weather Bureau. In the accompanying figures, precipitation is expressed as monthly departures from the 5-year average to show wet and dry periods.

Biological and physical data are shown in the accompanying graphs. Since appreciable numbers of the mosquitoes were detected only during the months of March through November data for this period only are shown. Mosquito population indices for the area were determined separately for the two principal species by averaging counts from the 17 stations.

OBSERVATIONS

Populations of *A. quadrimaculatus* were affected in general by the condition of the reservoir, which was the principal local breeding place of this species. When the water level remained close to the 5-year average, or slightly below, and when little summer fluctuation occurred, as in 1947 (Fig. 1) and 1950 (Fig. 4), the *A. quadrimaculatus* population was relatively small. Densities were much greater when the impoundment level was high, as in the springs of 1948 (Fig. 2) and 1949 (Fig. 3). The spring population of *A. quadrimaculatus* was probably depressed in 1949 by the prolonged cool weather which persisted longer than in 1948. Favorable climatic and reservoir conditions permitted populations to reach a higher level in 1949 than in 1948, however. Maximum populations in 1948 (Fig. 2) were observed mainly in early or midsummer, but in 1949 (Fig. 3) the peak was not reached until the latter part of the season. The level of the impoundment was high in the spring of 1948 (Fig. 2) and fell slowly until August as temperatures rose. Thereafter, the water level remained slightly higher than the average, with

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* Inactivated April 30, 1952.

minor fluctuations, through October. In the spring of 1949 (Fig. 3), the water level was again high and did not recede as far as the 1948 level. In contrast to conditions in 1948, a marked elevation of the reservoir occurred in the late summer of 1949. In 1951 the water level declined during the mosquito-breeding season to a point well below the average (Fig. 5). Counts of *A. quadrimaculatus* that year were larger, however, than during the two less productive seasons of 1947 (Fig. 1) and 1950 (Fig. 4). This is attributable to the rapid growth of vegetation in the margin of the reservoir.

No consistent effect could be ascribed to the maximum temperatures, which were approximately 100°F. Limiting effects of minimum temperatures were apparent in the spring and autumn. Minimum temperatures below 40°F. depressed breeding of anophelines. The greater prevalence of *A. crucians* in early spring and late fall suggests that this species is able to develop at lower temperatures than is *A. quadrimaculatus*.

Levels of the woodland ponds in which most breeding of *A. crucians* occurred varied with fluctuations in the adjacent reservoir and with local rainfall. Populations of *A. crucians* were high during the spring of 1947 (Fig. 1) when rainfall was above normal and in the early part of 1948 (Fig. 2) when the reservoir level was high. Densities of *A. crucians* were moderately large in 1949 (Fig. 3) when the water level was high, in spite of subnormal rainfall. The widespread drought of 1950 and 1951 (Figs. 4 and 5) was responsible for the great decline in level of the impoundment and for the disappearance of the nearby woodland ponds. Breeding areas for *A. crucians* were exceptionally restricted during this period.

DISCUSSION

The flat terrain in which the Santee Reservoir is located favored development of breeding conditions that are uncommon on impoundments with steeper shorelines. The extensive marginal plant growth that developed during recessions of the reservoir provided favorable breeding places. Reservoirs in the Tennessee Valley, described by Boyd (1949), are quite different from the Santee Reservoir. The extreme decline in the level of the Santee Reservoir during 1951 did not result in a proportionate decrease in the density of *A. quadrimaculatus*. Consistent with experience elsewhere, however, breeding increased with rising water levels in 1948 and 1949.

Detrimental effects of high atmospheric temperatures were undoubtedly modified by the humidifying influence of the many square miles of water surface in the neighboring reservoir. A rather critical minimum temperature of approximately 40°F. did control the appearance of anophelines in the spring and their disappearance in autumn. Bradley and Fritz (1945) observed that the greatest densities of *A. quadrimaculatus* occurred from spring until late summer in the isothermal zone which includes the present study area. During the 5 years of this investigation, some variation was noted in the occurrence of maximum mosquito counts. Overwintering of *A. crucians* in the larval stages, as noted by Frohne and Hart (1949),

EXPLANATION OF FIGURES

FIGS. 1–5: Weekly deviations of reservoir level from 5-year average, weekly maximum and minimum temperatures, monthly departures of rainfall from normal, and average weekly numbers of *A. quadrimaculatus* and *A. crucians* in 17 index stations.

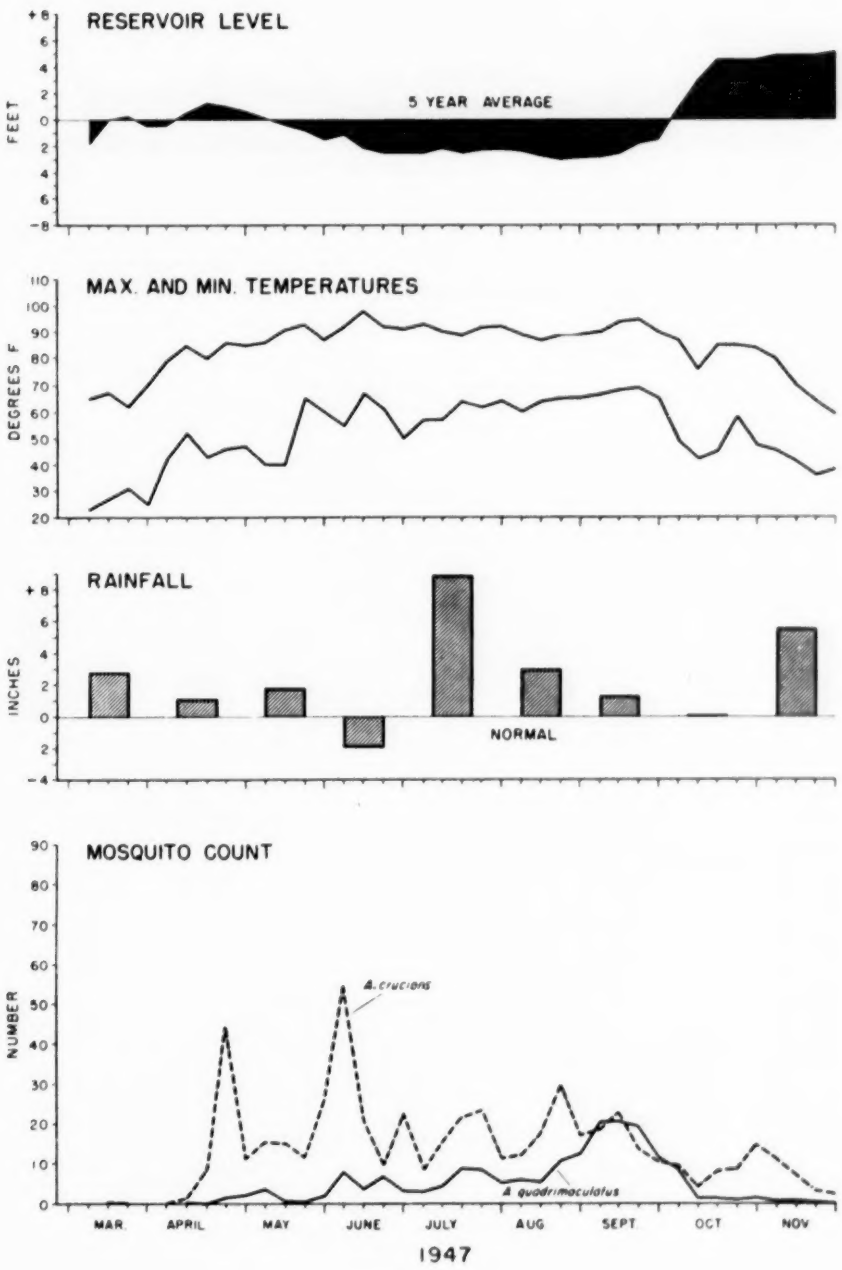
RESERVOIR LEVEL
FEET
+8
6
4
2
0
2
4
6
-8
5 YEAR AVERAGE
MAX. AND MIN. TEMPERATURES
DEGREES F
110
100
90
80
70
60
50
40
30
20
RAINFALL
INCHES
+8
6
4
2
0
2
-4
NORMAL
MOSQUITO COUNT
NUMBER
90
80
70
60
50
40
30
20
10
0
A. crucians
A. quadrimaculatus
MAR.
APRIL
MAY
JUNE
JULY
AUG
SEPT.
OCT
NOV
1947

FIG. 1.

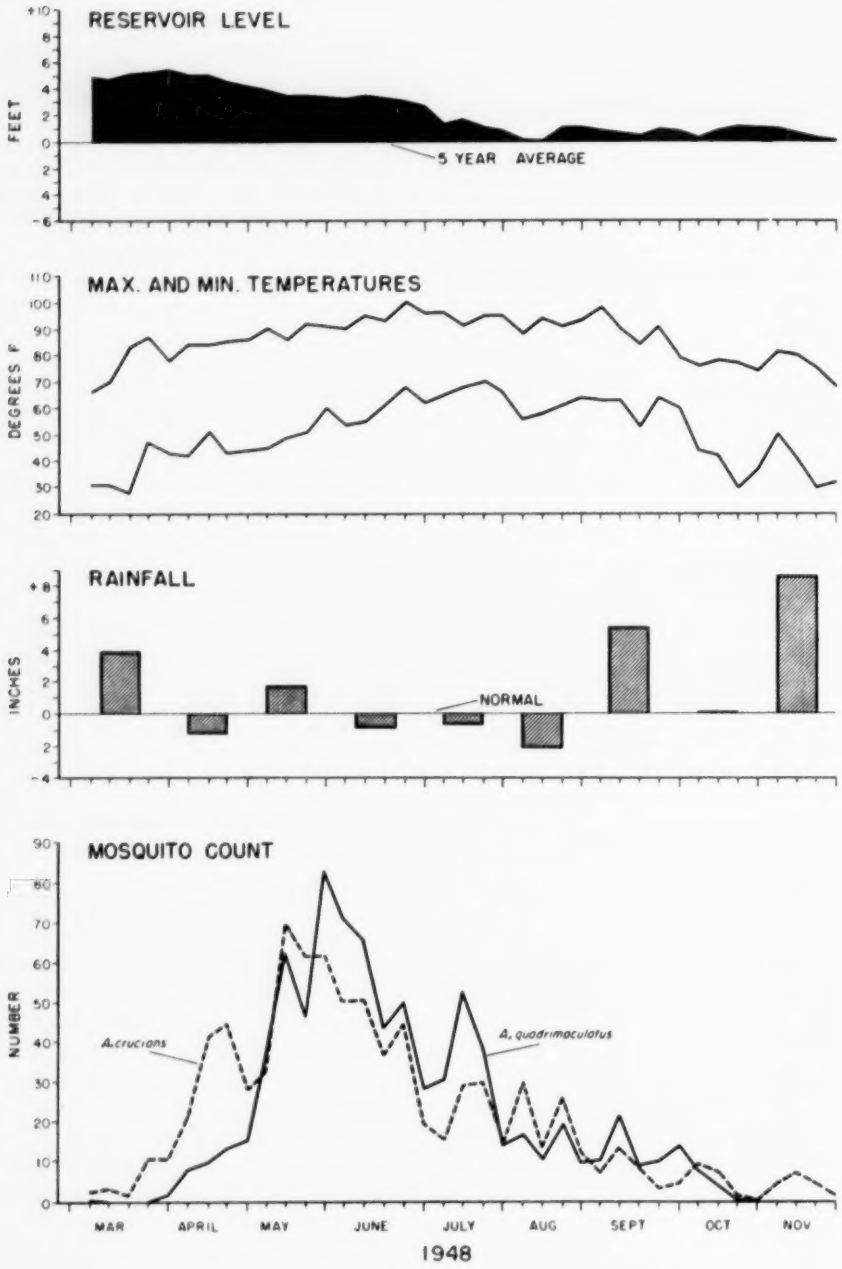
RESERVOIR LEVEL
FEET
+10
8
6
4
2
0
2
4
6
5 YEAR AVERAGE
MAX. AND MIN. TEMPERATURES
DEGREES F
110
100
90
80
70
60
50
40
30
20
RAINFALL
INCHES
+8
6
4
2
0
2
4
NORMAL
MOSQUITO COUNT
NUMBER
90
80
70
60
50
40
30
20
10
0
A. crucians
A. quadrimaculatus
MAR
APRIL
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
1948

FIG. 2.

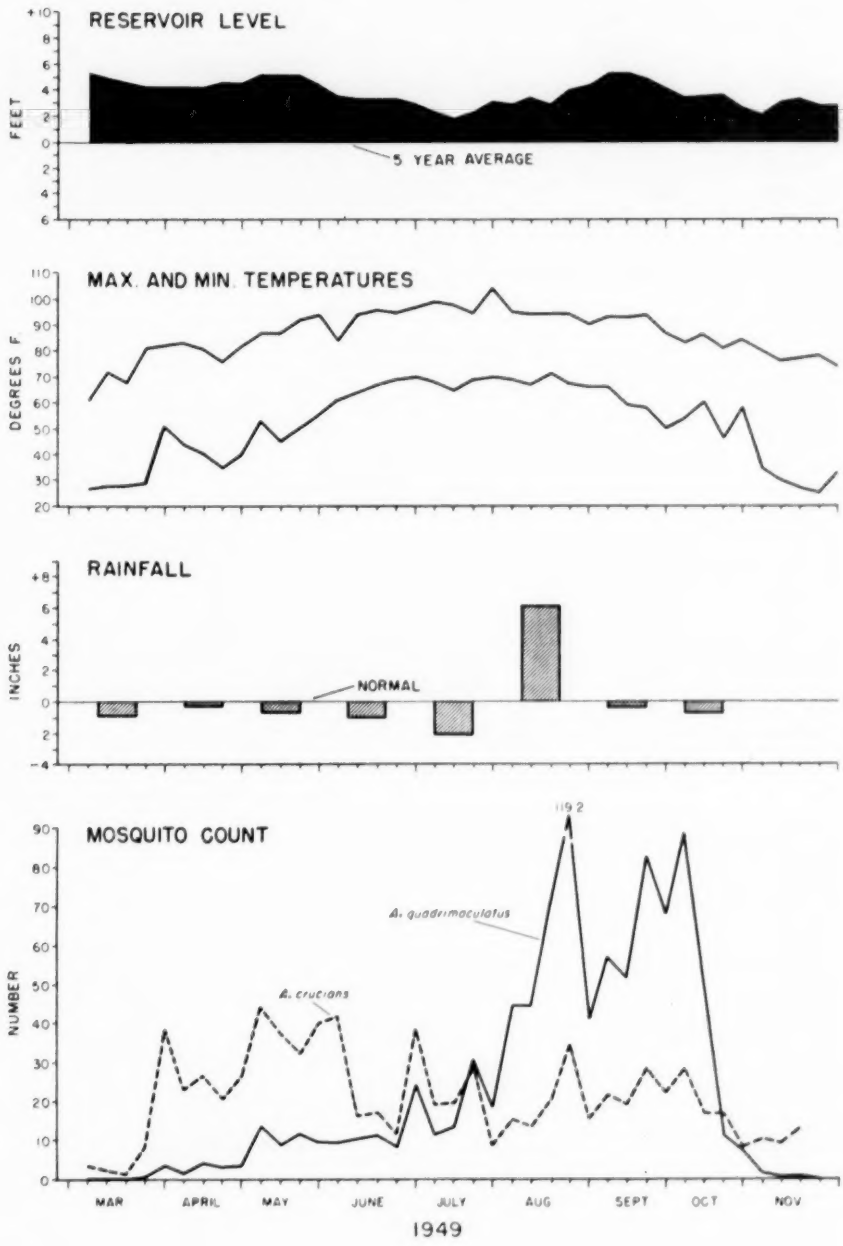
RESERVOIR LEVEL
FEET
+10
8
6
4
2
0
2
4
6
5 YEAR AVERAGE
MAX. AND MIN. TEMPERATURES
DEGREES F.
110
100
90
80
70
60
50
40
30
20
RAINFALL
INCHES
+8
6
4
2
0
2
4
NORMAL
MOSQUITO COUNT
NUMBER
90
80
70
60
50
40
30
20
10
0
119.2
A. quadrimaculatus
A. crucians
MAR
APRIL
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
1949

FIG. 3.

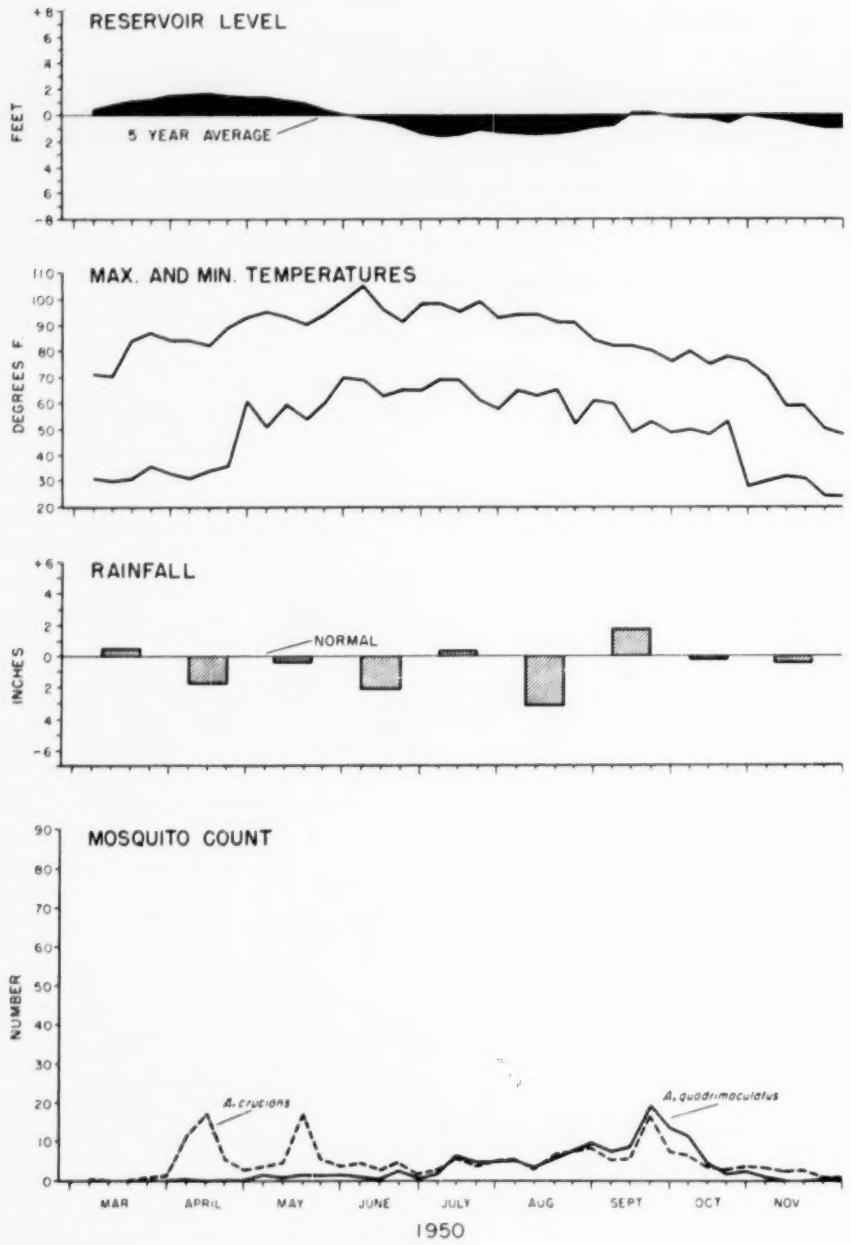
RESERVOIR LEVEL
FEET
+8
6
4
2
0
2
4
6
-8
5 YEAR AVERAGE
MAX. AND MIN. TEMPERATURES
DEGREES F
110
100
90
80
70
60
50
40
30
20
RAINFALL
INCHES
+6
4
2
0
2
4
-6
NORMAL
MOSQUITO COUNT
NUMBER
90
80
70
60
50
40
30
20
10
0
A. cruciens
A. quadrimaculatus
MAR
APRIL
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
1950

FIG. 4.

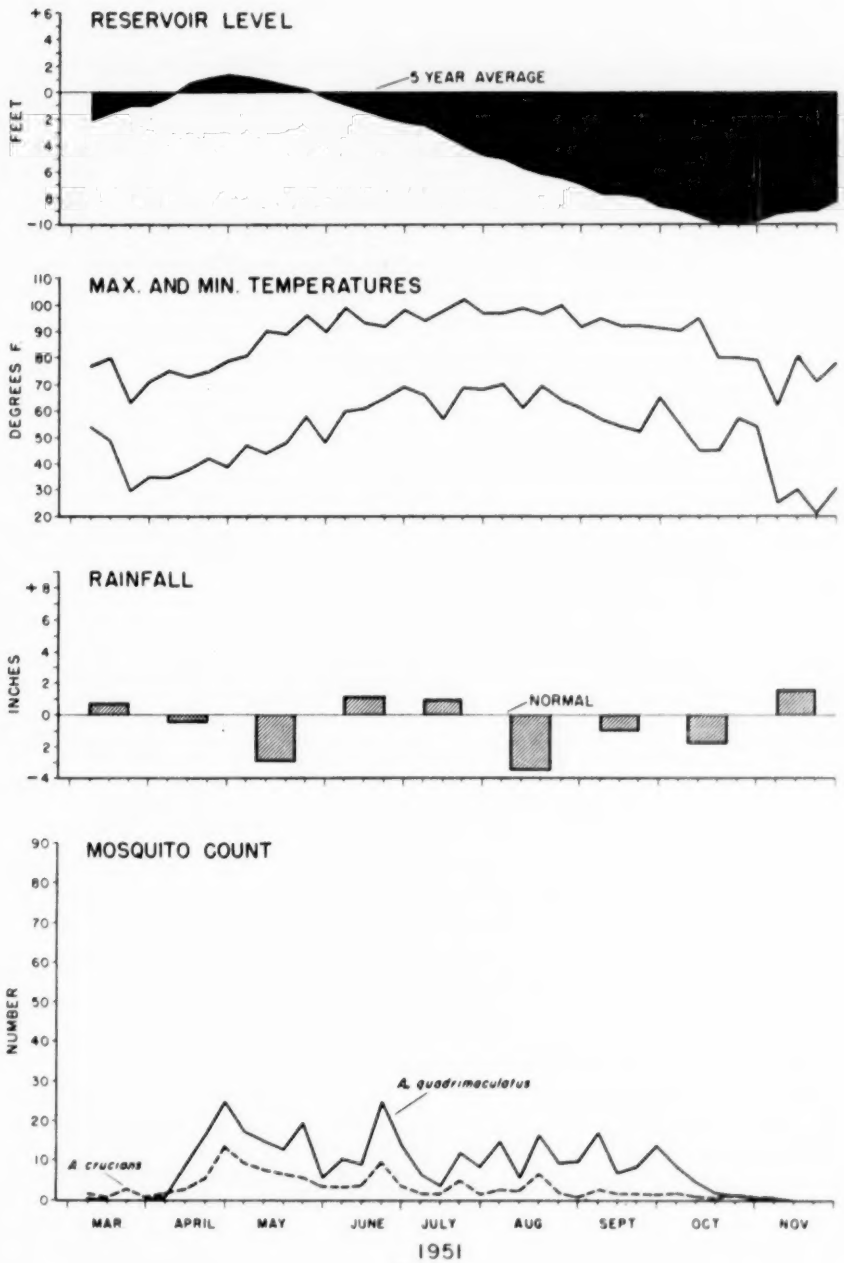
RESERVOIR LEVEL
FEET
+6
4
2
0
2
4
6
8
10
5 YEAR AVERAGE
MAX. AND MIN. TEMPERATURES
DEGREES F.
110
100
90
80
70
60
50
40
30
20
RAINFALL
INCHES
+8
6
4
2
0
2
4
NORMAL
MOSQUITO COUNT
NUMBER
90
80
70
60
50
40
30
20
10
0
A. quadrimaculatus
A. crucians
MAR
APRIL
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
1951

FIG. 5.

accounts for existence of adults of this species in significant number before *A. quadrimaculatus* adults appear in the spring.

Woodland pools adjacent to the reservoir gradually receded during the summer regardless of the local rainfall. This was particularly apparent in 1950 and 1951. The extreme recession of the impoundment in 1951 was attended by a drop in the water table in the neighboring territory.

CONCLUSIONS

Densities of adults of *A. quadrimaculatus* in a study area bordering the Santee Reservoir in South Carolina were related to conditions of the impoundment during a 5-year period, 1947–1951.

Densities of adults of *A. crucians* were influenced principally by levels of woodland ponds in this vicinity. Levels of these ponds were dependent upon height of the impoundment as well as upon local rainfall.

Temperatures above 40°F. apparently had no effect on densities of these anophelines, however temperatures below 40°F. reduced the density.

REFERENCES

BOYD, M. F. 1949 Malariology. Philadelphia and London. W. B. Saunders Co.

BRADLEY, G. H. AND FRITZ, R. F. 1945 Observations on seasonal occurrence and abundance of *Anopheles quadrimaculatus* Say. J. Nat. Mal. Soc. **4**: 251–261.

FROHNE, W. C. AND HART, J. W. 1949 Overwintering of *Anopheles crucians* Wied. in South Carolina. J. Nat. Mal. Soc. **8**: 171–173.

ADDITIONAL COLLECTIONS OF TICKS FROM SOUTHERN CALIFORNIA[1]

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The tick collections herein reported were obtained from mammals trapped during investigations concerned with insecticide studies for the control of ectoparasites under field conditions, and studies on the hosts of TRIATOMINAE. Twelve species of ticks were collected from five counties in southern California; for previous tick collections from this area the reader is referred to the works of Bishopp and Trembley (1945); Cooley and Kohls (1944, 1945); Cooley (1938, 1946); Davis *et al.* (1941), and Smith *et al.* (1946).

Many nymphs and larvae of *Ornithodoros* sp. collected from *Citellus beecheyi*, and *Ixodes* sp. from *Lynx rufus, Sylvilagus audubonii, Citellus beecheyi, Didelphis virginiana, Spilogale gracilis*, and *Neotoma fuscipes* are not reported because some of these immature ticks could not with certainty be identified to species.

The number of ticks indicated for each collection was obtained from one host unless otherwise indicated; a number greater than one in parentheses following the

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[1] This investigation was made possible by a contract (DA-49-007-MD-185) from the Research and Development Division, Office of the Surgeon General, Department of the Army, and by a grant (E-173) from the National Institutes of Health, U. S. Public Health Service, Department of Health, Education, and Welfare.

specific name of the host indicates the number of mammals in the pool from which the ticks were collected.

Reche Canyon is listed as being in both San Bernardino and Riverside Counties; this is not an error because Reche Canyon begins in Riverside County and enters the Santa Ana River in San Bernardino County.

The authors were assisted in this investigation by Charles T. Ames, Barbara A. Deem, Karl C. Fischer, Ken Y. Arakawa, and Christian P. Christianson; we also wish to acknowledge with appreciation the assistance of Glen M. Kohls of the National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana, for identification of the 12 species of ticks reported from five counties in southern California.

IXODIDAE

Ixodes pacificus Cooley and Kohls, 1943: *Canis latrans*, 2 miles north Etiwanda, San Bernardino County, March 13, 1952 (3-F†); *Camp Pendleton, San Diego County, March 27, 1953 (1-M) and April 3, 1953 (4-F); *Lynx rufus*, *Camp Pendleton, San Diego County, March 20, 1953 (1-F, 1-M), March 27, 1953 (8-F, 5-M†), and April 3, 1953 (1-F, 2-M); *Mustela frenata*, *Camp Pendleton, San Diego County, April 3, 1952 (1-F); *Urocyon cinereoargenteus*, Upper San Juan Camp, Riverside County, December 10, 1952 (4-F, 1-M).

Ixodes neotomae Cooley, 1944: *Neotoma lepida*, *San Juan Capistrano, Orange County, November 30, 1951 (1-F); *Sylvilagus audubonii*, *San Juan Capistrano, Orange County, November 30, 1951 (2-F, 6-M); *Dana Point, Orange County, December 8, 1952 (2-F), December 10, 1952 (2-F), and December 11, 1952 (2-F).

Ixodes texanus Banks, 1908: *Didelphis virginiana*, *Dana Point, Orange County, October 29, 1951 (1-F); *Mephitis mephitis*, *San Juan Capistrano, Orange County, October 4, 1951 (1-F, 3-N†); *Spilogale gracilis*, *San Juan Capistrano, Orange County, October 18, 1951 (1-F, 40-L†, 9-N) and *Dana Point, Orange County, October 22, 1952 (2-F, 50-L).

Ixodes woodi Bishopp, 1911: *Neotoma fuscipes*, **San Timoteo Canyon, Riverside County, January 8, 1952 (2-N); *Neotoma fuscipes* (nest), **San Timoteo Canyon, Riverside County, January 8, 1952 (1-F) and January 27, 1952 (1-L, 2-N).

Haemaphysalis leporis-palustris (Packard), 1869: This common tick was collected from *Sylvilagus audubonii* at San Juan Capistrano and Dana Point in Orange County; Reche Canyon, Riverside County; Loma Linda, San Bernardino County; and Camp Pendleton, San Diego County; *Lynx rufus*, Reche Canyon, San Bernardino County; *Citellus beecheyi*, San Juan Capistrano and Dana Point, Orange County; Camp Pendleton, San Diego County; and Loma Linda, San Bernardino County.

Dermacentor occidentalis Marx, 1892: *Citellus beecheyi*, Dana Point, Orange County, June 17, 1952 (1-N); *Neotoma fuscipes* (9), Mill Creek Canyon, San Bernardino County, March 17, 1953 (1-N).

Dermacentor variabilis (Say), 1821: *Canis latrans*, Camp Pendleton, San Diego County, March 20, 1953 (7-F, 31-M), March 27, 1953 (7-F, 17-M), and (3) April 3, 1953 (58-A, 2-F, 9-M). *Lynx rufus*, Camp Pendleton, San Diego County, March 20, 1953 (1-F, 1-M), (3) March 27, 1953 (66-A, 1-F, 8-M), and April 3, 1953 (7-F, 10-M); *Procyon lotor*, Camp Pendleton, San Diego County, March 16, 1953 (2-F); *Sylvilagus audubonii* (3), Camp Pendleton, San Diego County, July 24, 1952 (1-F, 1-M); *Taxidea taxus*, Camp Pendleton, San Diego County, March 20, 1953 (1-F, 5-M).

ARGASIDAE

Otobius lagophilus Cooley and Kohls, 1940: *Sylvilagus audubonii*, *Reche Canyon, Riverside County, October 10, 1951 (1-N).

Ornithodoros coriaceus Koch, 1844: Man, *Devil's Punch Bowl, Los Angeles County, August 21, 1952 (1-L, 1-N).

Ornithodoros parkeri Cooley, 1936: *Citellus beecheyi*, *San Juan Capistrano, Orange County, September 2, 1951 (1-M), (2) *Dana Point, Orange County, June 17, 1952 (3-N), and June 18, 1952 (1-F); Burrow of *Citellus beecheyi*, *Loma Linda, San Bernardino County, July 19, 1951 (5-F, 9-M, 20-N), July 23, 1951 (4-M, 10-N), *Dana Point, Orange County, August 19, 1951 (2-M), and *Camp Pendleton, San Diego County, August 26, 1952 (9-F, 22-M, 16-N).

Ornithodoros talaje (Guerin-Meneville), 1849: *Neotoma* (nest), *Victorville, San Bernardino County, July 13, 1953 (1-F).

* County Record, ** State Record.

† F—female, M—male, A—adult, L—larvae, N—nymph.

Ixodes texanus from Orange County differ from typical *I. texanus* females in that the former lack the prominent hump between the hypostome and the palpi; specimens in our collection are treated as *I. texanus* until additional studies indicate that a change in status is warranted.

Ixodes woodi taken in San Timoteo Canyon are larger than those previously examined from other collections (Texas); they are listed as *I. woodi* pending further investigation.

Ornithodoros parkeri was found in relatively large numbers and in all stages of development in the burrows of *Citellus beecheyi*, the California ground squirrel; at Loma Linda one burrow contained 34 ticks, many engorged when collected. Squirrels trapped at their burrows were parasitized by comparatively few ticks indicating that *O. parkeri* usually feeds on its host while the mammal is in the burrow.

SUMMARY

Twelve species of ticks (*Ixodes pacificus, neotomae, texanus, woodi; Haemaphysalis leporis-palustris; Dermacentor parumapertus, occidentalis, variabilis; Otobius lagophilus; Ornithodoros coriaceus, parkeri* and *talaje*) are reported from five southern California counties. These parasites were collected from fourteen genera of mammals, namely: *Lynx, Canis, Mustela, Mephitis, Spilogale, Urocyon, Procyon, Taxidea, Citellus, Lepus, Sylvilagus, Neotoma, Didelphis* and man. These tick collections constitute ten new county records and one new state record, i.e. *Ixodes woodi*.

LITERATURE CITED

BISHOPP, F. C. AND TREMBLEY, H. L. 1945 Distribution and hosts of certain North American ticks. J. Parasit. **31**: 1–54.

COOLEY, R. A. 1938 The genera *Dermacentor* and *Otocentor* (Ixodidae) in the United States, with studies in variation. Nat. Inst. Health Bull. (171), 89 pp.

——— 1946 The genera *Boophilus, Rhipicephalus* and *Haemaphysalis* (Ixodidae) of the new world. Nat. Inst. Health Bull. (187) 53 pp.

——— AND KOHLS, G. M. 1944 The Argasidae of North America, Central America and Cuba. Am. Midland Naturalist Monogr. Ser. (1) 152 pp.

———, and ———1945 The genus *Ixodes* in North America. Nat. Inst. Health Bull. (f84) 243 pp.

DAVIS, G. E., WYNNS, H. L., AND BECK, M. D. 1941 Relapsing Fever: *Ornithodoros parkeri* a vector in California. Pub. Health Rep. 56 (51) pp. 2426–2428

SMITH, C. N., COLE, M. M., AND GOUCK, H. K. 1946 Biology and control of the American dog tick. Tech. Bull. (905) U. S. Dept. Agr. 74 pp.

CHATIA BRENNAN, 1946: A NEW GENERIC CHARACTER AND ADDITIONAL NOTES (ACARINA: TROMBICULIDAE)

JAMES M. BRENNAN

The U. S. Department of Health, Education, and Welfare, Public Health Service,
National Institutes of Health, National Microbiological Institute,
Rocky Mountain Laboratory, Hamilton, Montana

The leeuwenhoekiine genus *Chatia* is monotypic for *Chatia setosa* Brennan, 1946, and is unique among the TROMBICULIDAE in that the tarsi of the larvae lack an empodium. The empodium is also absent in *Cockingsia, Grossia, Mackerrasiella,* and *Neotrombidium* which Womersley (1954) has tentatively placed in the APOLONIINAE. These, however, do not appear to be acceptable trombiculid genera. The larvae are parasitic only on arthropods, have only a single tarsal claw, and differ in certain other characters commonly associated with the TROMBICULIDAE.

In describing *Chatia* I overlooked the important taxonomic character of a ventral row of minute teeth on the cheliceral blade. This character has also been neglected by others (see references under redescription below). The teeth are not readily discernible except in full lateral view of the blade when their presence is unmistakable. In *C. setosa* the number of teeth that can be seen distinctly depends on how greatly the ventral margin of the blade is reflexed. In some specimens I have been able to count as many as 14 teeth of nearly uniform size.

I became aware of this condition when recently examining material from Utah, and later observed the character in specimens of the type series, including the holotype.

Now the genus may be redescribed as follows:

Chatia Brennan, 1946

Chatia Brennan, 1946, p. 132; Radford, 1947, p. 275; Ewing, 1950, pp. 293–294; Wharton *et al.*, 1951, p. 26; Wharton and Fuller, 1952, p. 101; Jameson and Toshioka, 1953, p. 89; Audy, 1954, pp. 163 and 165; Gould, in press, 195–.

Trombiculid larvae of the subfamily LEEUWENHOEKIINAE. Eyes present. Spiracles and tracheae absent. Cheliceral blade with tricuspid cap and a ventral row of minute teeth. All tarsi with 2 claws, empodium lacking. Scutum without anteromedian projection and with 3 pairs of setae of similar form: anterosubmedians, anterolaterals and posterolaterals; sensillae flagelliform.

Type species: Chatia setosa Brennan, 1946.

The characters of the multipronged palpal tibial claw and multisetose coxa III included in the original description have been omitted intentionally since, at best, they are probably of no more than specific value.

This finding of the cheliceral teeth provides a further character for separating *Chatia* and the related *Shunsennia* Jameson and Toshioka, 1953. I have carefully re-examined the two species included in the latter genus, a paratype of the type species, *S. tarsalis* J. and T. (fortunately with the cheliceral blades in lateral view) and the lectotype, type series and other specimens of *S. ochotona* (Radford). The blades have no dentition other than the usual tricuspid cap.

The large size (a fully-engorged larva exceeds a millimeter in length) and opaque white color permit reasonably accurate identification with the unaided eye of *Chatia setosa* in life. If one can judge from available records, the species is con-

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fined to the United States, west of the Rocky Mountains, and is not abundant. Records in addition to those reported in the original description from Ravalli County, Montana, and Elmore County, Idaho, extend its geographic distribution to Grays Harbor County, Washington, Plumas and Humboldt Counties, California, and Cache County, Utah. Specimens have been taken in the northern part of its range in all months from June to November, in California only in February (one record) and June. Host species recorded are *Tamiasciurus hudsonicus, T. douglasii, Eutamias townsendii, Citellus lateralis, Marmota flaviventer, Neotoma cinerea, Peromyscus maniculatus,* and *Ochotona princeps.*

A detailed redescription of *C. setosa* has been given by Gould (in press).

Summary

A neglected generic character, a ventral row of teeth on the cheliceral blade of the larva, is incorporated in the redescription of *Chatia* Brennan, 1946. Additional distributional notes are given for the genotype, *C. setosa.*

References

Audy, J. R. 1954 Malaysian parasites. IX. Notes on the taxonomy of trombiculid mites with description of a new subgenus. Stud. Inst. Med. Res. Malaya No. *26,* 1953, 123–170.

Brennan, J. M. 1946 A new genus and species of chigger, *Chatia setosa* (Trombiculidae, Acarina) from northwestern United States. J. Parasit. **32:** 132–135.

Ewing, H. E. 1950 A redescription of four genera of chigger mites, together wtih a description of a new genus and subgenus (Acarina, Trombiculidae). Proc. Ent. Soc. Wash. **52:** 291–299.

Gould, D. J. 195- The larval trombiculid mites of California. Univ. Calif. Publ. Entomol. In press.

Jameson, E. W. and Toshioka, S. 1953 *Shunsennia tarsalis,* a new genus and species of chigger from Korea (Acarina: Trombiculidae). Proc. Biol. Soc. Wash. **66:** 89–92.

Radford, C. D. 1947 New larval mites—Addenda. Proc. Zool. Soc. London, **117:** 275.

Wharton, G. W., Jenkins, D. W., Brennan, J. M., Fuller, H. S., Kohls, G. M., and Philip, C. B. 1951 The terminology and classification of trombiculid mites (Acarina: Trombiculidae). J. Parasitol. **37:** 13–31.

Wharton, G. W. aided by Fuller, H. S. 1952 A manual of the chiggers. Mem. No. 4, Ent. Soc. Wash., 185 pp.

Womersley, H. 1954 Malaysian parasites. VII. New genera and species, apparently of Apoloniinae (Acarina, Leeuwenhoekiidae) from the Asiatic-Pacific Region. Stud. Inst. Med. Res. Malaya No. *26,* 1953, 108–119.

KLOSSIELLA EQUI N. SP. (PROTOZOA: KLOSSIELLIDAE) FROM THE KIDNEY OF AN AMERICAN JACK*

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Protozoan parasites of the genus *Klossiella* have been reported in the mouse (*Klossiella muris*) by Smith and Johnson (1902) and in the guinea pig (*Klossiella cobayae*) by Seidelin (1914). Wenyon (1926) stated that a *Klossiella*, species undetermined, has been found in the kidney of the jerboa, a South African rodent. The purpose of this article is to describe *Klossiella equi*, n. sp., in the kidney of an American Jack, *Equus asinus*.

The host of the parasite was a jackass, 2½ years old, that died from spinal injuries incurred while he was being roped. Post-mortem examination revealed no gross evidence of any disease condition affecting the thoracic or abdominal viscera. Routine histologic examination was made of the brain, liver, and kidneys. Following the discovery of the sporogonic cycle of a protozoan parasite in the kidneys, a search was made for schizogonic forms in the capillary endothelial cells of the liver and brain, but none were found.

The parasite was found within epithelial cells lining the thick limbs of Henle's loops situated in the medullary rays of the kidneys. There were 4 morphological variations of the parasite apparently representing 4 stages in development. Three of the variations are designated by descriptive terms as follows: (1) uninuclear form, (2) multinuclear form, and (3) budding form. The fourth variation consisted of developing spores within the parasitized epithelial cell.

Description of the developing oocyst: (1) The uninuclear form (Fig. 1) was a round or slightly oval mass of reticulated protoplasm averaging 11 microns in diameter in formalin-fixed paraffin-embedded tissue. There was a small nucleus at the periphery, measuring approximately 3 microns in diameter and containing 1 or more small basophilic particles. Each parasite was located within a vacuole in the cytoplasm of its enlarged renal epithelial host-cell. The vacuole evidently was an artifact produced by shrinkage from fixing, dehydrating, and embedding the tissue for histologic section. (2) The multinuclear form was slightly larger than the uninuclear form, ranging from 18 to 22 microns in diameter. It consisted of a round or oval mass of reticulated protoplasm with multiple nuclei arranged along the border (Fig. 2). The nuclei were small, not very distinct and consisted of two small basophilic granules surrounded by a vaguely defined small area of condensed protoplasm. The epithelial cell containing the parasite was greatly enlarged and distorted, but not to such an extent that its identity was obscured. The multinuclear form differed from the uninuclear form in that it was slightly larger and that proliferation of the nucleus had occurred. (3) The budding form varied from 30 to 40 microns in greatest diameter; the smaller individuals were round and the larger ones ovoid. It was enclosed in a membrane apparently com-

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posed of the remnant of a greatly enlarged, parasitized renal epithelial cell. It consisted of a central mass of reticulated protoplasm covered on its entire surface by a single layer of contiguous budding processes. Each bud contained two small basophilic granules indicating the presence of a nucleus, but a boundary between nuclear and cytoplasmic substance within the bud could not be demonstrated. The buds were uniformly 3 to 4 microns in diameter, but varied in length (extent of projection from the central mass) depending on the size and degree of development of the entire organism. No nuclei were seen in the central protoplasmic mass. The appearance of the budding form varied according to the plane in which it was sectioned; those that were sectioned through the center showed the characteristic picture of a round mass of protoplasm with a ring of nucleated buds at the periphery (Fig. 3). On a morphological basis the budding form apparently represented a transitional stage of development between the multinuclear form and the young spore form. Observation of budding forms in different stages of growth gave the impression that the peripheral nuclei of the multinuclear form developed into the budding processes which eventually became somewhat elongated and were cast off to become young spores.

The fourth and final stage in development of the oocyst consisted of a group of spores enclosed in a thin walled sac (Fig. 4). The sac apparently was the remnant of a greatly enlarged parasitized renal epithelial cell and varied in size from 50 by 35 microns to 90 by 35 microns. The individual spores measured approximately 4 by 8 to 5 by 10 microns and were ovoid in shape.

An accurate count of spores in any sac could not be obtained; however 40 were found in cross section of one of the largest sacs observed. Although different stages of spore development were seen, all the spores in any particular sac were in the same stage of development. In the early stages (sporoblasts) there was no consistent pattern of protoplasmic structure, but nuclear division was apparent as indicated by a multiplicity of basophilic granules. As many as 4 double basophilic granules were seen in different locations in some of the spores. In the later stages a sporocyst wall developed that had a strong affinity for basic dyes and obscured the developing sporozoites within. However, a few sporocysts were so stained as to show sporozoites. Although the nature of the material did not permit an accurate count of the sporozoites, as many as 12 were counted in several sporocysts.

DISCUSSION

Illustrations and descriptions of *Klossiella* species by Wenyon (from unpublished data obtained from A. C. Stevenson) (1926), by Smith and Johnson (1902), and by Pearce (1916) show that structures corresponding to the 4 stages in development of the oocyst described above have been recognized by these authors and have been considered to represent stages in the sporogonic cycle. However, in view of the varied designations given to 3 of these forms by the different authors, it was considered advisable to employ designations based on morphology rather than on their presumed physiological state. The uninuclear form corresponds to Wenyon's illustration of a zygote and to Smith's illustration of sporonts and possibly to the hyaline intracellular forms described by Pearce. However, Figure 1 in the article by Pearce shows simple intracellular forms that correspond more closely to the uninuclear form than do her hyaline forms, but are not specifically mentioned in the legend or description of the parasite.

The multinuclear form corresponds to the sporont (or growing zygote) illustrated by Wenyon and to the mother sporoblasts illustrated and described by both Smith and Johnson and by Pearce.

The budding form corresponds to the "formation of sporoblasts" illustrated by Wenyon and to the "mother sporoblast with daughter sporoblasts" illustrated and described by Smith and Johnson. This form was not included in the sporogonic cycle by Pearce, who designated it the "ring" or "annular" form, and suggested that it may be the microgametocyte. However a progressive lengthening of the budding processes seen in our material strongly suggests that they are sporoblasts that eventually develop into spores.

The spore form is the final developmental stage of the parasite terminating in sporocysts containing sporozoites. The younger spores without recognizable sporozoites have been described by Smith and by Pearce as daughter sporoblasts and by Wenyon as sporoblasts that become enclosed within a sporocyst and produce sporozoites.

No stages in the schizogonic cycle were found. However Smith and Johnson found schizogony stages only in "certain heavily infected kidneys." Pearce found schizogony stages in the kidney of one of twelve infected guinea pigs. The kidney of the jack showed only a light to moderate infection.

In the opinion of the authors comparison of the parasitic forms in the kidney of the American Jack with published descriptions and illustrations of *Klossiella muris* and *Klossiella cobayae* warrant the conclusion that the parasite in the Jack is a new species of the genus *Klossiella* on the basis that the oocyst contained as many as 40 spores as compared with 12 to 16 spores for *K. muris* and 8 to 20 spores for *K. cobayae* according to Kudo (1946).

Klossiella equi, n. sp. (Figs. 1 to 4)

Description: Sporogonic cycle characterized by the development in the oocyst of as many as 40 ovoidal spores measuring 4 by 8 to 5 by 10 microns, each containing as many as 12 sporozoites. Schizogonic cycle unknown.

Habitat: Renal epithelial cells of the thick limb of Henle's loop.

Host: American Jack, *Equus asinus*.

Locality: Auburn, Alabama.

Types: U. S. N. M. Helm. Collection. Number 49,474.

SUMMARY

A protozoan parasite, *Klossiella equi*, spec. n., with developmental forms corresponding to the sporogonic cycle of *Klossiella muris* and *Klossiella cobayae* was found in the kidneys of an American Jack (*Equus asinus*).

REFERENCES CITED

KUDO, R. R. 1946 Protozoology, 3rd ed., Charles C. Thomas, Springfield, Ill. 778 pp.

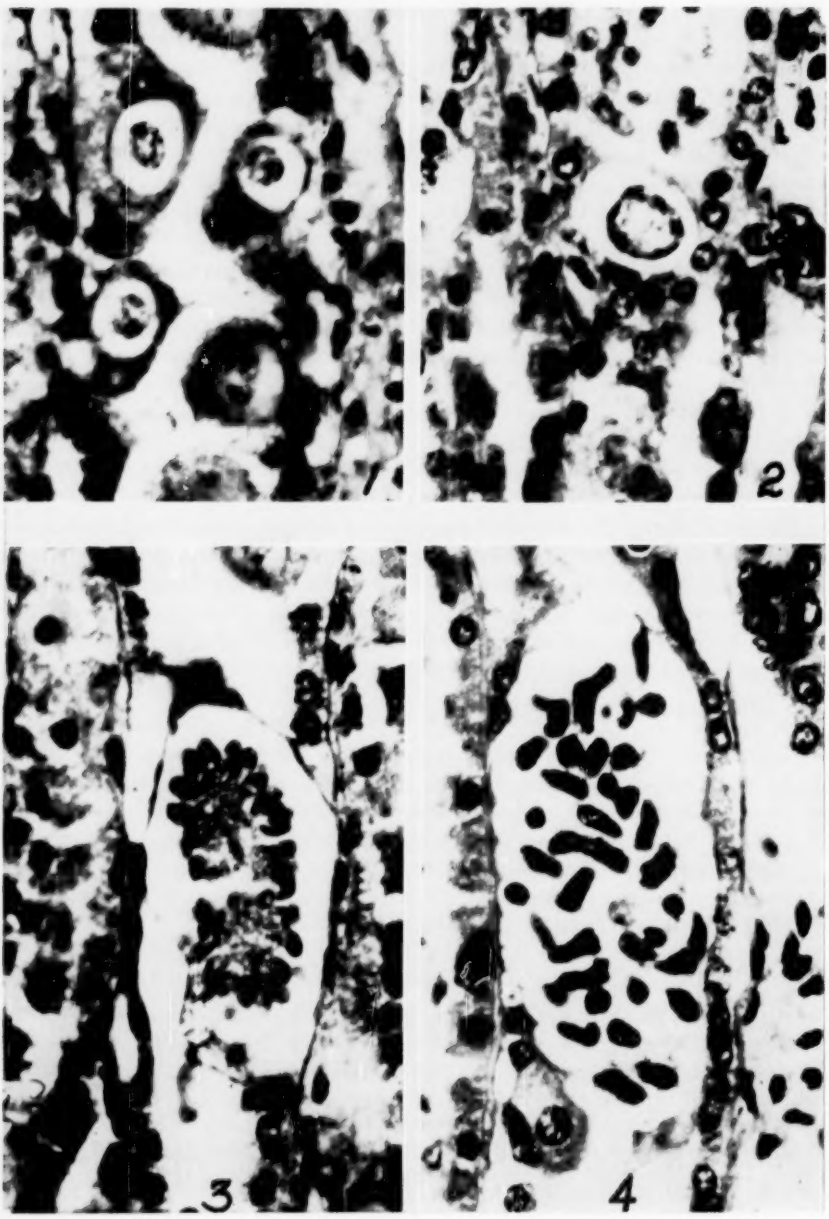
PEARCE, L. 1916 *Klossiella* infection of the guinea pig. J. Exp. Med. **23:** 431–442.

SEIDELIN, H. 1914 *Klossiella* sp. in the kidney of a guinea pig. Ann. Trop. Med. & Parasitol. **8:** 553–564. (Original reference not seen by authors.)

SMITH, T. AND JOHNSON, H. P. 1902 On a coccidium (*Klossiella muris*, gen. et spec. nov.) parasitic in the renal epithelium of the mouse. J. Exper. Med. **6:** 303–316.

WENYON, C. M. 1926 Protozoology; a manual for medical men, veterinarians and zoologists. V. 2, 779–1563 pp. Bailliere, Tindall and Cox, London.

PLATE I



EXPLANATION OF PLATE

FIG. 1. *Klossiella equi.* Developing oocyst. Unicellular form. 730×.

FIG. 2. *Klossiella equi.* Multinuclear form with nuclei arranged along the border. 730×.

FIG. 3. *Klossiella equi.* Budding form with round mass of protoplasm surrounded by a ring of nucleated buds. 730×.

FIG. 4. *Klossiella equi.* Oocyst with a group of spores enclosed in a thin wall sac. 730×.

A NEW SPECIES OF CHIGGER MITE FROM COLORADO (ACARINA, TROMBICULIDAE)[1, 2]

D. A. Crossley, Jr.

Among chiggers collected in southwestern Colorado, larvae of a species of *Euschöngastia* were found on three species of wood rats (*Neotoma*). This chigger, herein described as new, is an atypical member of the genus *Euschöngastia;* it appears related to *Euschöngastia lacerta* Brennan (1948) but differs in a number of characters.

The distinctive features of this mite were first recognized by Mr. L. J. Lipovsky, formerly of the University of Kansas. The author gratefully acknowledges the assistance of Mr. Richard B. Loomis and Dr. Charles D. Michener, University of Kansas, in the assembly and preparation of this paper.

The terminology used in the following description is essentially that of Wharton *et al* (1951) with some minor changes; see Audy (1952:152) for the use of tarsala and microtarsala in place of spur and microspur on the tarsi. Measurements are given in microns.

Euschöngastia finleyi new species

Diagnosis:—Larva having palpal claw three-pronged, sensilla sub-capitate, tarsus I without subterminala and parasubterminala, genuala III present, tibiala III present, six humeral setae, and sternal setae 2–4.

Description of larva:—

Body: Length and width of holotype 369 by 185 (engorged). Eyes 2/2, anterior eye larger; ocular plate poorly defined; distance across both eyes of one side 22 in holotype.

Gnathosoma: Capitular sternum with one pair of many-branched setae. Palpal femur and genu each with one branched seta; tibia with dorsal, lateral, and ventral branched setae; tarsus with tarsala (4.5 microns) and five (possibly six) feathered setae. Palpal claw with an apical prong and a pair of subapical, external accessory prongs. Cheliceral blade long, slightly curved, bearing a small tricuspid cap; basal segment about two-thirds as wide as long, lightly punctate basally. Galeal seta with one to five branches.

Scutum: Shape almost rectangular; anterior margin slightly emarginate above the anteromedian seta, posterior margin slightly emarginate laterad of the sensillary bases. Short, weak ridges anterior to sensillary bases. Sensillae sub-capitate (stem of sensilla widened immediately below the ovate head) with small stout barbs. Posterior portion of scutum lightly punctate. Standard measurements of holotype: AW—46, PW—60, SB—19, ASB—18, PSB—13, AP—25, AM—21, AL—26, PL—33, S—28. Averages and extremes of ten paratypes: AW—47 (45–50), PW—60 (57–66), SB—18 (16–20), ASB—19 (17–20), PSB—14 (12–16), AP—24 (22–26), AM—22 (18–25), AL—25 (23–28), PL—30 (28–33), S—28 (27–28).

Legs: Leg I coxa, trochanter, and basifemur each with one branched seta; telofemur with five branched setae; genu with one genuala, a microgenuala, and four branched setae; tibia with two tibialae, one microtibiala, and eight branched setae; tarsus with tarsala (10 microns), microtarsala, pretarsala, and about twenty branched setae, (no subterminala and parasubterminala). Leg II coxa and trochanter each with one branched seta; basifemur with two branched setae; telofemur with four branched setae; genu with one genuala and three branched setae; tibia with two tibialae and six branched setae; tarsus with an unusually long (15 microns) tarsala, microtarsala, pretarsala, and about fourteen branched setae. Leg III coxa and trochanter each with one branched seta; basifemur with two branched setae; genu with one genuala and three branched setae; tibia with one tibiala and six branched setae; tarsus with about thirteen branched setae.

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[1] Contribution No. 895 of the Department of Entomology, University of Kansas.

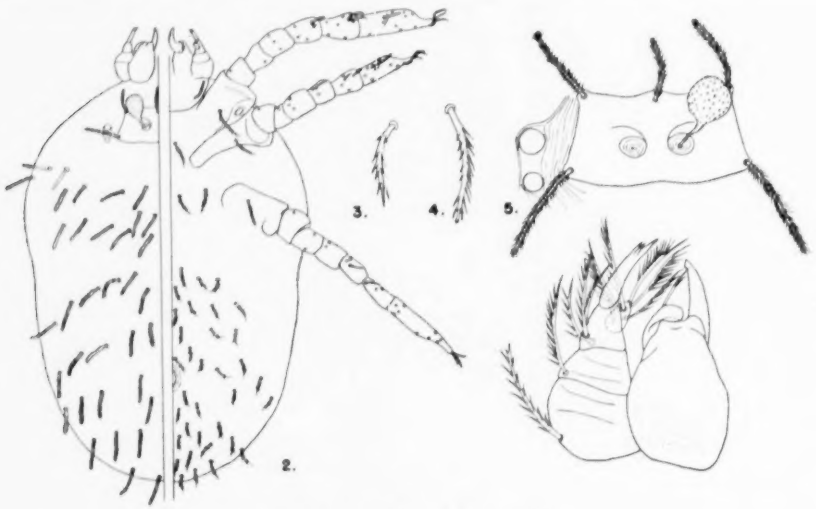
[2] The studies upon which this paper is based were conducted under a contract, N6 ori 220 Task Order II, between the University of Kansas and the Office of Naval Research.

Setae: Dorsal setal count difficult to formulate; approximately 6–8–4–10–12–10–10–6–2–2, total 70. Humeral setae extremely laterad and actually ventral on the specimens at hand (all engorged). Humeral seta measuring 28, anterior dorsal seta 23. Ventral setal formula 2–4–+ about 70; total about 76. Anterior ventral seta measuring 18.

Type material:—Holotype, KU slide number 7010, and two paratypes, KU 7011–7012, from *Neotoma cinerea*, female, field no. RBF491106-3; and two paratypes, KU 7013–7014, from *Neotoma mexicana*, female, field no. RBF491106-4, trapped 2.5 miles south of Fruita, 4600 ft., Mesa County, Colorado, on November 6, 1949, by Robert B. Finley, Jr. Also nine paratypes, KU 7015–7023, from 1.5 miles south of Loma, 4600 ft., Mesa County, Colorado, from *Neotoma lepida*, male, field no. RBF491107-3, taken on November 7, 1949, also by Robert B. Finley, Jr.

The holotype and paratypes are deposited in the Snow Entomological Museum, University of Kansas. Two paratypes will be sent to the United States National Museum and one paratype each to the Rocky Mountain Laboratory, Hamilton, Montana; the British Museum (Natural History); Dr. G. W. Wharton, University of Maryland; the South Australian Museum; and the Museum National d'Histoire Naturelle, Paris, France.

Remarks:—*Euschöngastia finleyi* possibly is related to *E. lacerta* Brennan (1948) and *E. "6"* n. sp. Farrell (in press); *finleyi* differs from these two species



Euschöngastia finleyi new species

FIG. 1, Dorsal aspect of body; FIG. 2, ventral aspect of body; FIG. 3, ventral body seta; FIG. 4, dorsal body seta; FIG. 5, scutum and eyes; FIG. 6, dorsal view of left side of gnathosoma.

in having six humeral setae instead of two, four setae in the second row of sternals instead of two, genualae of legs II and III present, and five branched setae on the palpal tarsus instead of four. The other species of *Euschöngastia* which lack the subterminala and parasubterminala are *E. enemi* Brennan and Jones (1954), *E. romala* Brennan and Jones (1954), and *E. "m"* n. sp. Gould (in press). These three species differ from *E. finleyi* in lacking a tibiala III; also, *E. enemi* has a 5-pronged palpal claw and a radically different scutum, and *E. romala* lacks a genuala III and has foliate dorsal body setae.

Loomis (1954) established the subgenus *Euschöngastoides* in the genus *Trombicula* for *T. hoplai* Loomis, 1954. It was suggested that *Euschöngastia lacerta* might belong to that subgenus despite the nature of the sensilla. The two species share several characters in common; namely, lacking subterminala and parasub-

terminala on leg I, lacking genualae on legs II and III, and having but four branched setae and a tarsala on the palpal tarsus. *Euschöngastia finleyi*, as well as *E. romala* and *E. enemi*, share the absence of subterminala and parasubterminala, but differ in the other characters of the subgenus. It seems best to leave the subgenus *Euschöngastoides* restricted to *T. hoplai* until a better evaluation can be obtained of the species lacking subterminala and parasubterminala.

SUMMARY

Described as new is *Euschöngastia finleyi*, a chigger mite parasitizing Colorado wood rats, *Neotoma* spp.

LITERATURE CITED

AUDY, J. R. 1952 Trombiculid mites infesting bats in Malaya with descriptions of three new species. Bull. Raffles Mus., Singapore, No. 24: 132–159, Nov., 1952.

BRENNAN, J. M. 1948 New North American chiggers (Acarina, Trombiculidae). J. Parasit. **34**: 465–478.

——— AND JONES, E. K. 1954 A report on the chiggers (Acarina, Trombiculidae) of the Frances Simes Hastings Natural History Reservation, Monterey County, California. Wasmann J. Biol. **12**: 155–194.

FARRELL, C. E. —— Studies on *Euschöngastia*, a genus of chigger mites, of North America. Proc. United States Nat. Mus., in press.

GOULD, D. J. —— The larval trombiculid mites of California. Univ. of California Pub. in Ent., in press.

LOOMIS, R. B. 1954 A new subgenus and six new species of chigger mites (genus *Trombicula*) from the central United States. Univ. Kansas Sci. Bull. **36**: 919–941.

WHARTON, G. W., JENKINS, D. W., BRENNAN, J. M., FULLER, H. S., KOHLS, G. M., AND PHILIP, C. B. 1951 The terminology and classification of trombiculid mites (Acarina: Trombiculidae). J. Parasit. **37**: 13–31.

INFULA MACROPHALLUS SP. NOV., A DIOECIOUS CESTODE PARASITIC IN THE BLACK-NECKED STILT, *HIMANTOPUS MEXICANUS*

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Two black-necked stilts collected, September 15, 1954, from a brackish lagoon about 10 kilometers from Salina Cruz, Oaxaca, Mexico, harbored two species of cestodes, *Hymenolepis himantopodis* (Krabbe, 1869) and three specimens of another species which are the subject of this paper. The birds were examined in the field immediately after being shot in order to obtain the helminths in the best possible condition. Sublimate-acetic fixative, Harris' haematoxylin, terpineol, and damar were used to prepare slides. The helminths were alive when removed from the hosts and provided excellent stained material. All measurements are in millimeters taken from specimens mounted in damar. This study was carried out while the author was a Muellhaupt Scholar in the Department of Zoology and Entomology, The Ohio State University.

The genus *Infula* was erected by Burt (1939) to include *I. burhini* from Charadriiform birds collected in Ceylon. He separated the genus *Infula* from

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Gyrocoelia mainly on the basis of the rostellar armature. It is apparent with *I. burhini* and the worm named here that the rostellum is not only unarmed, but differs in structure from that found in *Gyrocoelia*. In other respects the genera are very similar.

Infula macrophallus sp. nov.

Figures 1–8

(Measurements in millimeters)

Diagnosis: Female strobila larger and more powerful than male, as much as 145 long and 4.9 wide. Individual proglottids subtrapezoidal; anterior and posterior margins not parallel, with cirrus located on longer side. Proglottids of all ages broader than long, ratio changing with maturity. Scolex of female 0.53 in diameter, neck broader than long, 0.40 by 0.14. Suckers unarmed, from 0.25 to 0.27 by 0.32 to 0.36. Rostellum somewhat conical, unarmed, with fine fibers arranged like a sphincter near distal end. Musculature heavy, longitudinal bundles arranged in three layers. Innermost muscle bundles measuring 0.007–0.013, bundles of middle layer 0.006–0.010, muscles in third layer smaller and scattered more diffusely through cortical parenchyma. Fine, short transverse muscle fibers lying between longitudinal muscle bundles. Subcuticular muscles very fine. Longitudinal excretory vessels large (dorsal 0.022 and ventral 0.045), thick-walled and varying in size. Transverse commissures (dorsal 0.004 and ventral 0.007) in posterior part of proglottid connecting the longitudinal excretory vessels and taking the shape of a flattened "M". Genital pores lateral, irregularly alternating, near middle of proglottid. Genital sinus short and somewhat muscular. Terminal part of female genitalia (vagina) apparently identical to male cirrus-sac; externally with heavy muscular wall (0.004 thick) and covered with single layer of large (glandular ?) cells; internally with glands cells and typical armed duct possessing an enlargement containing material which might be confused with spermatozoa. Vagina passing between longitudinal excretory canals, distal part of vagina eversible. Proximal vagina minute, thin-walled, coursing to tubular, arcuate seminal receptacle (?) lying adjacent and anterior to vitelline gland; spermatozoa absent in specimens studied here. Seminal receptacle (?) possessing incomplete septa. Proximal vagina and seminal receptacle (?) apparent only in very young proglottids. Female system beginning development early; primordium of vagina appearing first. Ovary disappearing when uterus partially fills with eggs. Young uterus an annular tube of irregular diameter extending almost to margins of ovary and vitelline gland. Enlarging uterus with numerous diverticula forming externally and fewer internally. Eggs discharged from uterus through mid-dorsal and mid-ventral pores located on inside of velum. Uterine pore perhaps with small sphincter. Uterus often almost emptied; dorsal to ovary. Vitelline gland ventral, reniform in frontal section, with part of large Mehlis' gland located in concavity. Uterine passage connecting oötype to young uterus at anterior midpoint. Eggs (in whole mounts) generally collapsed. Embryonic hooks 0.015 to 0.019 long. Numerous nuclei possessing prominent nucleoli and heavy peripheral chromatin situated in central portion of embryo. One flame cell observed in this region.

Male strobila smaller than female strobila, as much as 34 long and 1.47 wide. Scolex 0.50 in diameter, suckers 0.18 to 0.22 by 0.22 to 0.25. Neck broader than long, 0.36 by 0.15. Rostellum unarmed and similar to that of female. Proglottids similar in shape to those in female strobila, but becoming almost as long as broad with age. Testes 35 to 50, reaching diameter of 0.080 by 0.093, in medullary parenchyma. Longitudinal excretory vessels thick-walled, 0.006 and 0.016, varying in diameter. Transverse commissures with diameter of 0.004 and 0.010, similar in shape and disposition to those in female. Young cirri with armed portion distended to 0.055 while still in cirrus-sac. With age, this portion becoming reduced in size. Uneverted, mature cirrus measuring 0.14–0.18; smaller than vagina of female, but otherwise similar. Internal seminal vesicle large, containing many spermatozoa. Vas deferens swollen, functioning as external seminal vesicle. Both seminal vesicles ephemeral.

Host: Black-necked stilt (*Himantopus mexicanus*).

Locality: Near Salina Cruz, Oaxaca, Mexico.

Site of infection: Small intestine.

Type specimens: Holotypes, male and female, in Helminthological Collection of U. S. National Museum, No. 37470.

Infula macrophallus can be differentiated readily from *I. burhini* by the possession of larger embryonic hooks, fewer testes, and larger scolices and suckers. The erection of this genus by Burt on the basis of the absence of rostellar hooks

may well be questioned, because it is known that hooks frequently drop off rostella in moribund specimens. However, in this instance, Burt claimed that his specimens were in good condition. The tapeworm described by me was fixed while still motile. Examination of the rostellum with a phase contrast microscope revealed neither scars (where hooks had been) nor muscles (which had been attached to hooks). Furthermore, an examination of specimens of *I. burhini* deposited in the National Museum by Marietta Voge and C. P. Read established still more firmly the unarmed nature and the conical, rather than capitate, shape of the rostellum (Fig. 4) in this genus. In the closely related genus *Gyrocoelia*, the rostellum is capitate, even in moribund specimens. The description of a new species here and the work of Voge and Read (1954) remove most of the doubt concerning the validity of the genus *Infula*.

The method of copulation among the dioecious tapeworms and in those lacking a complete vagina is poorly understood. Burt (1939) called, what is structurally the same as the cirrus in the male, a vagina in the female, because of its probable function. He reported spermatozoa in this structure and in the seminal receptacle, and he interpreted this as evidence that copulation had taken place. In the female strobilae studied here, no sperm were observed in any of the segments before the formation of eggs. In these young segments the developing vagina possesses a large lumen distally before many cells have proliferated between the outer muscular wall and the armed portion. The proximal vagina is very delicate and courses along the aporal side of the ovary and uterus to a sausage-shaped seminal receptacle (?). This latter structure disappears a short time after it is first apparent and the proximal part of the vagina is not apparent in the more mature proglottids. The presence of these structures (proximal portion of the vagina, the seminal receptacle (?), and the distal vagina with a large lumen) concomitantly in young proglottids, before eggs have formed, is strong evidence for interstrobilar copulation. However, the absence of spermatozoa in these specimens is very puzzling.

ACKNOWLEDGMENT

The author wishes to acknowledge here the prompt loan by Allen McIntosh of specimens of *I. burhini* which are located in the National Museum.

LITERATURE CITED

BURT, D. R. R. 1939 On the cestode family Acoleidae, with a description of a new dioecious species, *Infula burhini* gen. et sp. nov. Spolia Zeylanica **21**: 195–208.

VOGE, M. AND READ, C. P. 1954 A new record of the cestode *Infula burhini* Burt (Cyclophyllidea: Dioecocestidae) from Australia. J. Parasit. **40**: 483.

EXPLANATION OF PLATE

All figures except Fig. 4 were drawn with the aid of a microprojector and concern *Infula macrophallus*.

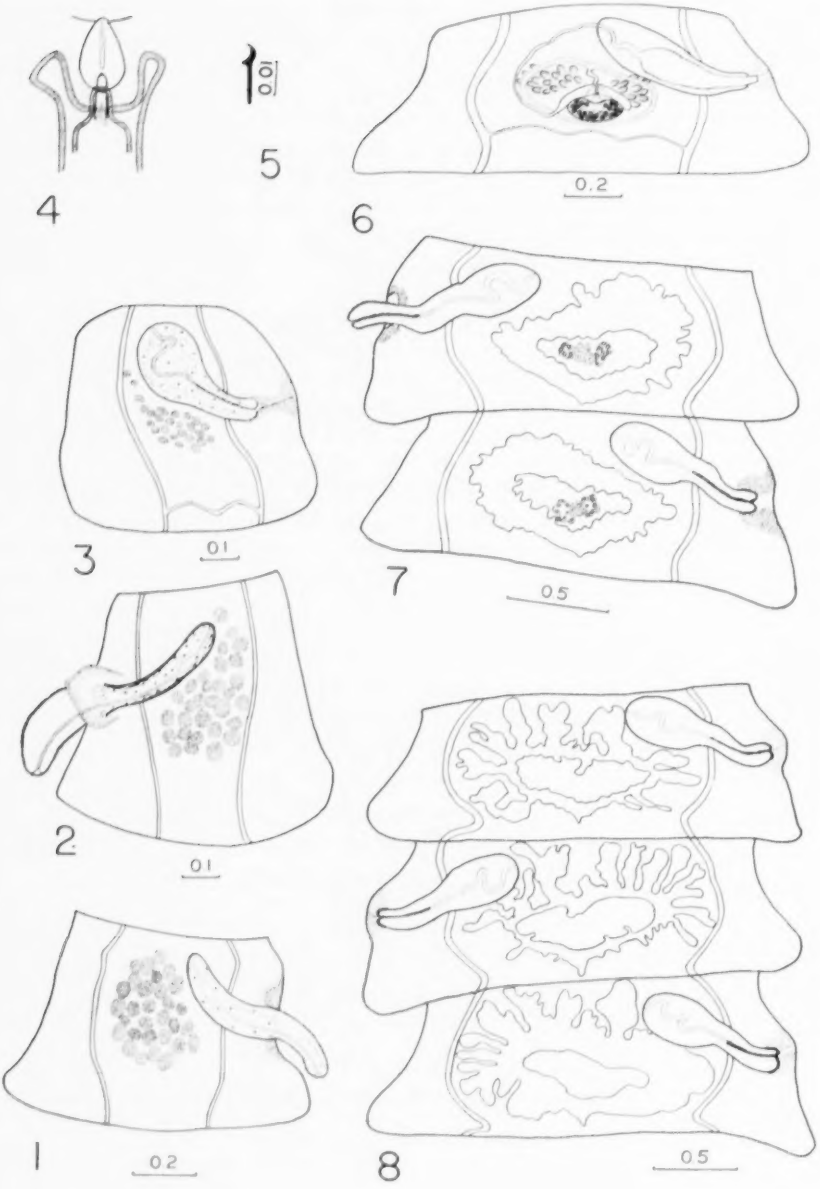
FIGS. 1–3. Proglottids of various ages from the male strobila.

FIG. 4. Rostellum and excretory ducts of *Infula burhini* from specimens in U. S. National Museum Collection. Freehand sketch.

FIG. 5. Embryonic hook.

FIGS. 6–8. Proglottids of various ages from the female strobila.

PLATE I



MICROAGGLUTINATION REACTIONS OF *TRICHOMONAS SUIS, T.* SP., AND *T. FOETUS**

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Atrophic rhinitis of swine has become a disease of great interest in recent times. The etiology has been studied from many viewpoints by various workers. Switzer (1951) described trichomonads in the nasal passages of 80 per cent of swine affected with atrophic rhinitis. He also reported them to be present in only 2.8 per cent of swine not affected with the disease. He further made morphological studies which showed that this nasal form was not entirely like *Trichomonas suis* which was mentioned by Davaine (1877). The nasal form described by Switzer (1951) may have been the same as the trichomonads reported from a facial lesion on a pig by Hegner and Alicata (1938). In an abstract Hammond and Fitzgerald (1953) reported further studies on the trichomonads in the pig and added some information on forms found in the stomach. Their studies were morphological and cultural in nature. They reported morphological and cultural differences among the three forms and noted that the stomach form was more similar to *T. suis* from the cecum than it was to the nasal form. Spindler, Shorb and Hill (1953) indicated with studies on experimental infections the possibilities of the nasal form and *T. suis* from feces being the same or similar.

Since immunological studies have proved efficacious in the description of certain protozoa, it was felt that there might be advantage in such procedures with the forms of *Trichomonas* from the hog. Riedmüller (1932), according to Trussell (1947), used guinea pigs to build up complement-fixing antibodies by intra-peritoneal injection of vaginal discharge containing *T. vaginalis*. Agglomerating and killing antibodies were demonstrated by Tokura (1936), as reported by Trussell (1947), using rabbits and formaldehyde killed cultures of *T. vaginalis, T. hominis,* and *T. buccalis*. At low dilutions Tokura noted some group antigen indications, but cross reactions showed specificity in higher dilutions. Morgan (1944) made a study of agglutinins against *T. foetus* in the sera of different vertebrates. The technique employed was similar to that of Trussell (1946) in microagglutination tests on the sera of rabbits immunized against *T. vaginalis*.

MATERIAL AND METHODS

In the present study three trichomonads were used. They were *Trichomonas suis* from the cecum of a hog affected with atrophic rhinitis, *T.* sp. from the nasal passages of the same hog, and *T. foetus*. Protozoa for the study were counted with a haemocytometer.

Seven rabbits were utilized for antibody production. The rabbits were inoculated with living cultures of the trichomonads in C.P.L.M. (cystein–peptone-

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liver–maltose) medium once a week for a period of eleven weeks. The dosage was as follows with noted exception: inoculum #1, 2.5 million; inoculum #2, 6.5 million; inocula #3 through #6, 6 million; inocula #7 and #8, 10 million; inoculum #9, 15 million; inocula #10 and #11, 20 million. The eighth inoculum given rabbit 3 with *T. foetus*, consisted of 1.5 million due to an unexplained difficulty in growing the cultures at that time. The organisms for inoculation were grown in C.P.L.M. medium after Trussell (1946). Rabbits 7 and 8 were injected with C.P.L.M. This plain medium was washed in the same manner as were the various cultures, with M/15 NaH_2PO_4, and the amount injected was the average volume of inoculum used in the inoculations of trichomonads at that time.

In general the antigen for agglutination tests was prepared after the method and with the modified C.P.L.M. medium described by Trussell (1946). However, the final concentration of test antigen was made up to two million organisms per

TABLE 1.— *Agglutination titers of trichomonads formalinized and suspended in Ringer's solution or M/15 NaH_2PO_4 with various antisera and a normal serum control.*

Serum number code: 1 and 2—*Trichomonas* sp. (pig nose)
3 —*T. foetus*
5 and 6—*T. suis*
7 and 8—C.P.L.M. control

Antigen	Serum	Titer		Control Titer	
		NaH_2PO_4	Ringer's	NaH_2PO_4	Ringer's
T. sp.	1	640	640	40	10
	2	640	320	40	10
	3	20	30	40	10
	5	40	40	20	10
	6	40	20	40	10
	7	160	10	40	10
	8	80	20	20	10
T. suis	1	160	160	40	80
	2	40	80	40	80
	3	160	80	40	80
	5	640	320	40	80
	6	640	320	40	80
	7	160	40	40	80
	8	80	40	40	80
T. foetus	1	160	40	40	10
	2	80	20	40	10
	3	1280	640	160	10
	5	160	160	40	10
	6	80	80	40	10
	7	40	40	40	10
	8	80	40	40	10

cc. This was found to be a more effective concentration of these forms which are smaller than *T. vaginalis*. Duplicate agglutination series were run using two different suspending media: namely M/15 NaH_2PO_4 and Ringer's solution. The test organisms were formalinized to a final concentration of 0.3 per cent formalin.

Blood was taken by heart puncture from rabbits 1, 2, and 3, four days after the last inoculation, from rabbits 5 and 6, three days after the last inoculation, and from rabbits 7 and 8, six days after the last inoculation. Serum was filtered through a Seitz DK.–8 filter, inactivated at 56 degrees C. for 25 minutes, and then stored in sterile serum bottles at 4 degrees C.

Hog sera for agglutination tests were collected with a bull bleeding 20 cc. syringe and a 3 inch 18 gauge needle. The serum was inactivated at 56° C. for 25 minutes and preserved by the addition of phenol to a final concentration of 0.3 per cent. The agglutination procedure was nearly the same as that described by Trussell (1946). The main difference was that the serial dilutions of the sera were made directly with the suspended organisms and M/15 NaH_2PO_4 as described by

Trussell (1946). Normal serum controls were run concurrently with all the test mixtures. The agglutination reactions were read at a magnification of 100×, using both dark field and phase contrast. Wide field oculars were definitely advantageous. The titer was established as the highest dilution of serum in which a few small clumps occurred (Trussell 1946).

RESULTS

The results of the agglutinations with the rabbit sera are shown in tabular form.

Agglutination tests were run on sera from hogs showing outward symptoms of atrophic rhinitis against *T.* sp. suspended in NaH_2PO_4. It was not possible to autopsy these animals, so conclusive diagnosis of the disease was not obtained, nor was it possible to ascertain whether or not trichomonads were present. Nine suspect sera were run along with one control serum. The control animal was autopsied and was not rhinitic nor did it harbor trichomonads.

The results showed only 4 sera with higher titers than the controls. Three of these were 160, and one was 80. Two other hog sera showed titers of 40, which was the same as the control titer in rabbit sera against *T.* sp. suspended in NaH_2PO_4. The other three hog sera showed no reaction and the control serum gave a titer of only five.

DISCUSSION

While the titers obtained in the agglutination tests using rabbit sera were not quite as high as those obtained by Trussell (1946) with *T. vaginalis*, they were high enough and distinct enough to give quite a clear antigenic difference. Titers and cross titers of the two hog trichomonads showed some antigenic difference. They were as different from each other as each was different from *T. foetus*. Ringer's solution was used also as a suspending medium for the antigen in addition to the NaH_2PO_4 buffer suspension suggested by Trussell (1946) because of the several good electrolytes it contains. The variation and instability of titers in Ringer's solution seemed to suggest that perhaps the profusion of different ions had a detrimental effect on the experiment. In the case of *T. suis*, Ringer's solution suspension, as compared to phosphate buffer solution suspension, definitely affected the agglutinations so as to raise the titer consistently in the control serum. It was not determined which ion or combination of ions did this.

The agglutination titers in phenolized serum from possible atrophic rhinitis affected hogs were not entirely consistent. However, there was enough consistency of titers to postulate the presence of a circulating antibody in the blood of hogs affected with atrophic rhinitis and harboring trichomonads. More work using serum without preservative would be desirable.

CONCLUSIONS[1]

Agglutination tests utilizing rabbit sera have shown definite antigenic differences between the two strains of trichomonads from the hog. These strains were isolated

[1] Since this work was done, a more thorough study of the antigenic properties of *T. vaginalis* and *T. foetus* has been made by Menolasino and Hartman (1954). Their work has revealed a complex antigenic structure of these protozoa and the production of several different antibodies in rabbits immunized against somatic and flagellar antigens. These workers concluded that *T. vaginalis* and *T. foetus* are similar antigenically. In the light of these new findings, it is felt that further study would serve to give a clearer picture of the relationship between *T. suis* and *T.* sp. (nose of rhinitic pig).

from the nose and cecum respectively of the same hog which was affected with atrophic rhinitis. This indicates that these two trichomonads may be different species. Further agglutination tests with hog sera demonstrated the possibility of the presence of nasal trichomonad antibodies in the blood of 4 out of 9 hogs showing symptoms of atrophic rhinitis.

BIBLIOGRAPHY

DAVAINE, C. J. 1877 Traité des entozoaires et des maladies vermineuses de l'homme et des animaux domestiques. 2nd ed., 1003 pp., Paris.

HAMMOND, D. M. AND FITZGERALD, P. R. 1953 Observations on trichomonads of the digestive tract and nose of pigs. J. Parasit. **39** (4 sec. 2): 11.

HEGNER, R. AND ALICATA, J. E. 1938 Trichomonad flagellates in facial lesions of a pig. J. Parasit. **24:** 554.

MENOLASINO, N. J. AND HARTMAN, E. 1954 Immunology and serology of some parasitic protozoan flagellates. I. *Trichomonas vaginalis* and *Trichomonas foetus*. J. Immunol. **72:** 172–176.

MORGAN, B. B. 1944 Normal agglutinins in vertebrate sera for *Trichomonas foetus* (Protozoa). Proc. Helminth. Soc. Washington **11:** 21–23.

RIEDMÜLLER, L. 1932 Zur Frage der ätiologischen Bedeutung der bei Pyometra und sporadischem Abortus des Rindes gefundenen Trichomonaden. Schweiz. Arch. Tierheilk. **74:** 343–351.

SPINDLER, L. A., SHORB, D. A., AND HILL, C. H. 1953 The role of trichomonads in atrophic rhinitis of swine. J. Am. Vet. Med. Ass. **122:** 151–157.

SWITZER, W. P. 1951 Atrophic rhinitis and trichomonads. Vet. Med. **46:** 478–481.

TOKURA, N. 1936 Biologische und immunologische Untersuchungen über die menschenparasitären Trichomonaden. Zentralbl. Bakteriol., I. Abt., Ref. **121:** (7–8): 149.

TRUSSELL, R. E. 1946 Microagglutination tests with *Trichomonas vaginalis*. J. Parasit. **32:** 563–569.

———1947 *Trichomonas vaginalis* and trichomoniasis. 277 pp. Charles C Thomas, Springfield.

EFFECTIVENESS OF ABBOTT'S INSECT REPELLENT CREAM (E 4856) AS A PROTECTIVE BARRIER AGAINST *SCHISTOSOMA MANSONI* IN MICE

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The need for an inoffensive preparation which can be easily applied to the skin and will serve as an effective barrier to the penetration of schistosome cercariae is self-evident. Protection experiments with chemical barriers including repellent ointments and impregnated clothing have been studied with increased interest since the outbreak of World War II. Various investigators (Churchill 1945; Churchill and Wright, 1945; Nolan, Mann and Churchill, 1947; Kuntz, Stirewalt and Buchheit, 1947; Wright, Bauman and Fry, 1948a, 1948b; Hunter, *et al.*, 1949; Hunter, *et al.*, 1952; Kaufman, Hunter and Pan, 1949; Ritchie, *et al.*, 1952) have reported varying degrees of efficiency in numerous compounds against penetration of *Schistosoma japonicum* and *S. mansoni* cercariae. Although many of the ointments have resulted in a high degree of protection against schistosome cercariae in experimental animal tests, none is without some undesirable quality. The present paper reports

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the results of a series of tests that were performed to determine the protective quality of Abbott's Insect Repellent Cream (E 4856) against penetration of *S. mansoni* cercariae in experimental mice.

MATERIALS AND METHODS

The experimental cream is an odorless, colorless preparation containing Rutgers 612—2%, Indalone—2% and Dibutyl Phthalate—6% in a plasticized compound of silicone, nitrocellulose and castor oil in a vanishing cream base. A single application on the skin repels water for many hours but is readily removed by repeated washings with soap and water. The animals used were young male and female mice reared from the stock laboratory strain maintained in the Tropical Research Medical Laboratory, San Juan, Puerto Rico. The cercariae used were *S. mansoni* harvested from naturally infected *Australorbis glabratus* (Say) recently collected from an endemic area in Puerto Rico. Cercariae from 25 infected snails were combined and the pooled harvest was estimated by counting three aliquots of the suspension, and determining the number of cercariae per milliliter. The desired number of cercariae to be used per exposure was transferred to a Kahn serum tube with a 3 ml. automatic pipette. All animals were exposed to cercariae by the tail immersion method (Wright, *et al.*, 1948b; Olivier and Stirewalt, 1952). The test ointment was applied to the animal's tail and massaged with the finger tips until a thin, even coating covered the entire skin surface. Excess ointment was removed with a damp cotton sponge. The mice were held in restraining tubes for at least 30 minutes to 1 hour, with the tail projecting through a one-hole rubber stopper into the cercarial suspension.

Three series of experiments were conducted, utilizing 230 mice. *Series 1:* A single application of the test ointment was given to groups of mice 1, 6, 12 and 24 hours before the animals were exposed to 150 *S. mansoni* cercariae. Those exposed after 1 and 6 hours were held in continuous restraint without opportunity to lick or in any other manner clean their tails. Those exposed 12 and 24 hours after application of ointment were returned to their cages (groups of 5 each) and allowed normal activity except during exposure to the cercariae. *Series 2:* Four groups of mice in this series were exposed to only 100 cercariae by the same technique 24, 48, 72 and 96 hours after a single application of the test cream. *Series 3:* After a single application of the test ointment, mice were placed in restraint each day and their tails allowed to soak in test tubes filled with clear tap water for a period of one hour (the last soaking was one hour before exposure to cercariae). Groups of mice were exposed to 100 cercariae 72, 120, 168 and 240 hours after a single application of the repellent cream. An appropriate number of control mice was put through identical manipulations and exposed to each group of cercariae but the application of the experimental repellent-cream was omitted.

Surviving animals were sacrificed 6–8 weeks after exposure to cercariae. Adult schistosomes were recovered by perfusion and the remaining worms were obtained by teasing the tissues apart. Microscopic examination of the entire viscera and lungs was made between glass plates with the aid of a stereoscopic microscope to detect immature forms as well as eggs in the various organs. When possible all dead mice not surviving the 6–8 week period were autopsied and all worms were enumerated.

RESULTS

Series 1: All mice exposed to 150 cercariae of *S. mansoni* 6 hours or less after a single application of the experimental ointment were completely free of schistosomal infection. Fifteen adult worms were recovered from 1 of 15 treated mice which were exposed 12 hours after a single application of the test cream; while from a corresponding control group of 10 mice an average of 67.0 (± 20.9) adult schistosomes were recovered. Three of the 15 mice exposed 24 hours after the test cream was applied were completely free of infection; the average number of recovered adult schistosomes was again consistently lower in the treated group (18.9 ± 20.9 vs 79.4 ± 17.6) and in only one treated mouse did the number of adult worms exceed the minimal number in the control group. *Series 2:* The mice in this series were exposed to 100 cercariae. The interval of 24 hours between the application of the ointment and cercarial exposure, as used above, was repeated and extended to 48, 72 and 96 hours. All the mice in the group exposed after 24 hours

TABLE 1.—*Summary of results obtained in testing Abbott's Insect Repellent Cream (E 4856) on mice as a protective barrier against* S. mansoni *cercariae.*

Intervals between treatment and exposure	Cercariae per mouse	Treated mice						Control mice					
		Deaths in 4-8 wks. period	No. mice examined	No. mice positive	% totally protected	Adult worms recovered		Deaths in 4-8 wks. period	No. mice examined	No. mice positive	% totally protected	Adult worms recovered	
						Mean	S.D.					Mean	S.D.
30–60 min.	150	0/ 9*	10	0	100	0	—	7/10	10	10	0	88.8	± 34.0
6 hrs.	150	0/15	15	0	100	0	—	5/10	9	9	0	60.7	± 14.3
12 hrs.	150	1/15	15	1	93	1.0	—	6/10	10	10	0	67.0	± 20.9
24 hrs.	150	1/15	15	12	20	18.9	± 20.9	8/10	8	8	0	79.4	± 17.6
24 hrs.	100	0/10	10	10	0	12.3	± 5.2	4/ 5	5	5	0	51.2	± 8.8
48 hrs.	100	3/10	10	10	0	22.5	± 9.3	1/ 5	5	5	0	30.0	± 5.8
72 hrs.	100	0/10	10	10	0	64.8	± 24.7	5/ 5	5	5	0	72.4	± 15.6
96 hrs.	100	5/10	10	10	0	34.6	± 11.8	5/ 5	5	5	0	45.0	± 8.2
72 hrs. with 3 daily soakings	100	3/10	9	8	11	20.5	± 16.1	1/ 9	8	8	0	54.6	± 8.1
120 hrs. with 5 daily soakings	100	4/10	6	6	0	44.0	± 19.9	3/10	8	8	0	40.3	± 18.3
168 hrs. with 7 daily soakings	100	3/10	10	10	0	45.5	± 11.2	2/10	10	10	0	39.1	±11.2
240 hrs. with 10 daily soakings	100	2/10	8	8	0	48.3	± 12.8	5/ 5	4	4	0	53.7	± 11.4

* One mouse died 3 days after exposure to cercariae. No evidence of infection at autopsy.

were infected but the largest number of worms found in a treated mouse was 21, while 40 was the smallest number in a control animal. The average number of worms found was also much less than in the controls, 12.3 ± 5.2 compared to 51.2 ± 8.8. The average number of worms found in the mice exposed 48, 72 and 96 hours after a single application of ointment was lower in each experimental group than in the controls but it was apparent that there was little or no protection (See Table 1). *Series 3:* Four groups of mice in this series were also exposed to 100 cercariae. During the intervals of 72, 120, 168 and 240 hours between the application of the ointment and the exposure to cercariae, the tails were soaked daily for 1 hour in water. One of 9 mice autopsied in the first experimental group was negative for worms, and the average number found in these mice was much less than in the controls, 20.5 ± 16.1 compared to 54.6 ± 8.1. The greatest number of worms found in the experimental group was 39, while in the controls the smallest number was 45. There was no evidence of any protection in the other three groups

of experimental animals in this series. A summary of the results obtained will be found in Table 1.

CONCLUSIONS

Under certain conditions, Abbott's Insect Repellent Cream (E 4856) served as an effective barrier against penetration of cercariae of *S. mansoni* in mice when applied to the skin surface. The protection was essentially absolute for 12 hours and was marked over a 24 hour period. Evidence of protection persisted in one series of animals for 3 days despite hour-long soakings in water on three consecutive days. There was no apparent protection after 72 hours.

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REFERENCES

CHURCHILL, H. M. 1945 Experiments to determine the effectiveness of agents applied to the skin in protection against schistosomiasis, OSRD., CMR. Trop. Diseases Report No. 67, pp. 1–4.

———, AND WRIGHT, W. H. 1945 Test on fabrics and ointments for the control of schistosomiasis, OSRD, CMR. Trop. Diseases Report No. 59, pp. 1–7.

HUNTER, G. W., III, BENNETT, H. J., FRY, N. H., SEE, J. AND GREEN, E. 1949 The control of schistosomiasis japonica. V. Studies on the penetration of various types of unimpregnated uniform cloth by cercariae of *Schistosoma japonicum*. Am. J. Trop. Med. **29:** 723–737.

———, RITCHIE, L. S., TANABE, H., NAGANO, K., PAN, C., AND YOKOGAWA, M. 1952 Studies on schistosomiasis. V. Testing protective ointments against schistosomiasis by using schistosome dermatitis producing cercariae. Tr. Roy. Soc. Trop. Med. and Hyg. **46:** 201–206.

KAUFMAN, E., HUNTER, G. W., III, AND PAN, C. 1949 Protection experiments with copper oleate ointment against schistosomiasis. J. Parasit. **35** (6-Sect. 2) : 29.

KUNTZ, R. E., STIREWALT, M. A., AND BUCHHEIT, J. R. 1947 Method for testing ointments and fabrics to determine their effectiveness as barriers to schistosome cercariae. Am. J. Trop. Med. **27:** 691–697.

NOLAN, M. O., MANN, E. R., AND CHURCHILL, H. M. 1947 The protective value of chemically impregnated fabrics against penetration of schistosome cercariae. Nat. Inst. Health Bull. 189, pp. 180–198.

OLIVIER, L., AND STIREWALT, M. A. 1952 An efficient method for exposure of mice to cercariae of *Schistosoma mansoni*. J. Parasit. **38:** 19–23.

RITCHIE, L. S., HUNTER, G. W., III, NAGANO, K., AND LIN, S. 1952 Studies on schistosomiasis. IV. Two ointments for protection against schistosomiasis japonica. Mil. Sur. **111** (2) : 106-110.

WRIGHT, W. H., BAUMAN, P. M., AND FRY, N. 1948a The control of schistosomiasis japonica. VI. Studies on the chemical impregnation of uniform cloth as a protection against schistosomiasis japonica. Am. J. Hyg. **47:** 33–43.

———, ———, AND ———1948b The control of schistosomiasis japonica. VII. Studies on the value of repellents and repellent ointments as a protection against schistosomiasis japonica. Am. J. Hyg. **47:** 44–52.

———, AND CRAM, E. B. 1948 Wartime research in human schistosomiasis. Proc. Fourth Internat. Cong. Trop. Med. and Malaria, Washington **2:** 997–1009.

STUDIES ON DRUG RESISTANCE IN COCCIDIA

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The addition of chemicals to poultry feed for preventing and controlling coccidiosis outbreaks has been widely and intensively applied in the United States during the past 6 years. This development has permitted the poultry-industry to expand greatly and has resulted in more efficient and economical production of poultry. Because of the variability of coccidiosis epidemics, and the difficulties encountered in treatment of outbreaks, continuous medication has become established practice to prevent morbidity and mortality losses in raising broilers and flock replacement chickens.

Although this method has been of great economic importance, it has not been ideal from the standpoint of therapeutics. This is because the dosage which is effective for "average" conditions may be sub-curative for "severe" coccidiosis and eventually permit the emergence of resistant strains of coccidia. Even before the time of Ehrlich's (1907) fundamental discoveries on drug-resistance in trypanosomes, it was known that bacteria and protozoa could be adapted to grow and survive in normally toxic concentrations of chemicals by repeatedly exposing the organisms to sub-lethal and increasing concentrations of the compounds. Drug-resistance did not become an important practical problem, however, until the development and wide-spread use of the sulfonamides and antibiotics. Later, after the extensive use of certain insecticides, it was found that flies, mosquitoes and other insects had become resistant to these chemicals. Obviously, such changes must occur if the organisms are to succeed in their struggle for survival. Furthermore, it is a well-known biological fact that all organisms are capable of adaptation. Whether these adaptive processes are genetic, enzymic, physico-chemical, or other, they may result in a "resistant" strain which is able to survive and reproduce in its environment.

Sulfaquinoxaline had been in continuous and satisfactory use since 1947 for controlling coccidiosis in a typical New England poultry farm and there was no evidence that drug-resistance had developed in the coccidia. Nevertheless, early in 1951, we began laboratory studies to determine whether resistance could be induced in coccidia by repeated exposure to sub-optimal dosages of certain anticoccidial agents. A short time later, studies were also begun on field strains of coccidia. These were obtained from poultry farms where, due to intercurrent diseases and other difficulties, anticoccidial medication appeared less effective than normally.

To our knowledge, the only report of studies on resistance in coccidia is the recent publication by Harwood and Stunz (1953). They found that a strain of cecal coccidia obtained from poultry houses in which nitrofurazone had been used for 3 years was fully susceptible to this drug. Likewise, they reported that cecal coccidia from an area where sulfaquinoxaline had been employed for about 4 years were fully susceptible to this anticoccidial agent. It was concluded that there was no evidence of drug-fastness in these coccidia.

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This report presents the results of our studies on the resistance found in field strains of coccidia from chickens and on induced resistance in laboratory strains of *Eimeria acervulina* and *Eimeria tenella*.

MATERIALS AND METHODS

All chicks used in these studies were obtained from commercial hatcheries when 1-day old. White Leghorns or New Hampshire Reds of both sexes were used. In most experiments, the groups were initially balanced for number, weight and sex. In the laboratory experiments, groups of 5 to 10 chicks each were kept in metal, wire-floored, battery brooders in isolation quarters. The experiment in floor pens was conducted under practical conditions in a broiler house using shredded cane litter and allowing 1 square foot per bird.

The water and feed were provided *ad libitum*. The feeds used were an open-formula laboratory ration composed of natural feed stuffs supplemented with all known required vitamins and open-formula commercial chick starter and broiler rations. The commercially available anticoccidial drugs were thoroughly mixed into the feeds just prior to use.

The strains of coccidia included pure populations of *Eimeria acervulina* and *Eimeria tenella* (Pennsylvania strain) isolated in our laboratory and *E. tenella* (Oregon strain) obtained from Dr. E. M. Dickinson, Oregon State College. In addition, mixed cultures of coccidia were recovered from 40 field samples submitted from various areas of the United States. The samples usually consisted of portions of the intestines and ceca and their contents. These materials were homogenized with an electrically driven blender and placed in 2.5% potassium dichromate solution for sporulation. If, after incubation, straining, washing and concentrating, there were insufficient sporulated oocysts in the suspension for direct use, the inoculum was administered in graded doses to groups of 5 chicks and the oocysts were recovered 7 and 8 days later. The numbers of oocysts in the original or passage suspensions were adjusted to contain 50,000 sporulated oocysts per ml.

The experiments to determine the sensitivity of the field strains of coccidia to the various drugs were performed by a standardized prophylactic procedure. Three-week old chicks, in groups of 5 or 10, were fed graded dosages of the medicated feeds 24 hours before and for 7 days after inoculation with 50,000 sporulated oocysts. On the 8th day the surviving chicks were weighed and sacrificed and the coccidial lesions were scored. The intestinal or cecal homogenates were prepared and oocyst counts were made as described below. All of these criteria were used in assessing the response of the strains to medication, but the most sensitive indicator was the oocyst count. It is important to note that, under these laboratory conditions of relatively severe infection, the dosages of drugs required for preventing mortality, decreasing the severity of the coccidial lesions, inhibiting oocyst producton and permitting optimal growth are 2- to 4-fold greater than the use-concentrations in the field. However, this relationship between anticoccidial effectiveness under laboratory and field conditions is relative because the dosage required for maximal effectiveness is proportional to the severity of infection.

The studies on the laboratory strains were conducted as follows. In the first experiment, *E. acervulina*, one of the non-fatal intestinal species, was selected for study because it has a short life-cycle, the oocysts sporulate quickly and it is the

most sensitive to sulfaquinoxaline of the various species occurring in chickens. It was planned to administer sulfaquinoxaline medicated feeds, at ¼ and ½ of the minimal concentration which inhibited oocyst production under our standardized laboratory conditions, to coccidia infected chicks for 5 serial passages. Challenge studies would then be conducted to determine whether the sub-strains had changed in sensitivity to sulfaquinoxaline. The serial passages were made with 8 or 9 day old chicks in groups of 10 each. The basal or medicated feed was available 24 hours before oral inoculation with 200,000 sporulated oocysts of *E. acervulina* obtained from stock culture or preceding sulfaquinoxaline exposed passage material. On the 5th day post-inoculation, the chicks were sacrificed and the entire small intestine was removed, brought to standard volume with 2.5% potassium dichromate solution and homogenized with a blender.

Subsequent, but similar, experiments were run with 2 strains of cecal coccidia, *E. tenella*. In addition to sulfaquinoxaline, studies were made with nitrophenide, nitrofurazone and nicarbazin, a recently described anticoccidial agent (Cuckler, Malanga, Basso and O'Neill, 1955). The assays were conducted with 2-week old chicks in groups of 10 each. The chicks were fed the medicated diets 24 hours prior to oral inoculation with 5,000 sporulated oocysts of *E. tenella*. Medication was given continuously for 8 days at which time the ceca were removed and homogenized for counting and sporulating the oocysts.

The following procedure was used for estimating the number of oocysts produced. Appropriate aliquots (5 ml.) of the intestinal or cecal homogenate were placed in tubes containing normal sodium hydroxide (5 ml.). After 2 or 3 hours the suspensions were "cleared" sufficiently by the sodium hydroxide to permit rapid and accurate count of the oocysts present. Each suspension was thoroughly mixed and 4 separate aliquots were removed with a wide-bore pipette and placed on a hemacytometer by the usual technique. The number of oocysts was counted for each of 4 volumes of 0.5 cmm. If the individual counts varied from each other by more than ± 20%, another series was counted. The total number of oocysts found was multiplied by the dilution factor (100,000) and divided by the number of chicks sacrificed in the group to obtain the number of oocysts per chick. The balance of the homogenate was strained and placed in shallow pans and incubated for 3 to 5 days. It was then washed to remove dichromate and the numbers of oocysts were adjusted for the succeeding serial passage.

EXPERIMENTAL RESULTS

Field Strains

Coccidia from chickens were obtained from 40 sources in 14 different States during a period of 27 months. These coccidia were studied because of alleged "poor" performance of anticoccidial medication. Various medication procedures and products had been used prior to our obtaining samples of the coccidia. In many instances intercurrent diseases, other than coccidiosis, were also present. Most of these strains of coccidia were studied for sensitivity to sulfaquinoxaline, nitrophenide and nitrofurazone and, later, to nicarbazin. The results of these studies are summarized in Table 1.

Seventeen (43%) of the 40 allegedly resistant strains studied were normally sensitive to the various anticoccidial drugs. Resistance to nitrophenide was en-

countered in 13 strains (43%). Nine of these were cross-resistant to sulfaquinoxaline and nitrofurazone and 4 to nitrofurazone. None of the strains was resistant only to nitrophenide or to sulfaquinoxaline and nitrophenide. Sulfaquinoxaline resistance was found in 18 strains studied (45%). Six of these strains were resistant to sulfaquinoxaline only; 3 to sulfaquinoxaline and nitrofurazone; and 9 to sulfaquinoxaline, nitrophenide and nitrofurazone. The highest rate of resistance (57%) was found for nitrofurazone which was ineffective for 17 of the strains. One strain was resistant to nitrofurazone, but sensitive to the other anticoccidial

TABLE 1.—*Effect of Nicarbazin, Nitrofurazone, Sulfaquinoxaline and Nitrophenide on field strains of Coccidia*

Strain no.	Location	Type of coccidiosis[1]	Response to Medication[2]			
			0.02% Nicarbazin	0.025% Nitrofurazone	0.05% Sulfaquinoxaline	0.05% Nitrophenide
1	Delaware	I & C	–	S	S	S
2	New York	I & C	–	S	S	S
3	"	C	–	S	S	S
4	Georgia	I & C	–	S	S	S
5	"	C	–	S	S	S
6	Maryland	I & C	S	S	R	S
7	Connecticut	C	S	S	R	S
8	Massachusetts	I & C	–	R	S	R
9	Delaware	C	S	R	S	R
10	Maryland	C	S	R	R	R
11	"	C	S	R	R	R
12	"	C	S	R	R	R
13	Oregon	C	S	S	S	S
14	Delaware	I & C	S	R	S	R
15	Massachusetts	C	S	R	R	R
16	Delaware	C	S	S	S	S
17	Maine	C	–	–	S	–
18	"	C	–	–	S	–
19	Connecticut	C	S	S	R	S
20	Maine	I & C	–	–	S	–
21	Maryland	C	S	R	R	R
22	"	C	S	R	R	R
23	Pennsylvania	I	–	–	S	–
24	Delaware	C	S	R	S	R
25	Connecticut	C	–	–	S	–
26	Maryland	C	S	R	R	R
27	Maryland	I & C	–	–	S	–
28	New Jersey	I & C	S	–	S	–
29	Pennsylvania	I & C	S	R	S	S
30	Texas	I & C	S	–	S	–
31	Delaware	I & C	S	S	R	S
32	"	I & C	S	S	R	S
33	Maryland	I & C	S	R	R	S
34	Virginia	I & C	S	R	R	R
35	Delaware	C	S	S	R	S
36	Texas	I & C	S	R	R	S
37	Maine	I & C	S	–	S	–
38	Indiana	I & C	S	–	S	–
39	Ohio	I & C	S	R	R	S
40	Delaware	I & C	S	R	R	R
	No. examined		28	30	40	30
	No. resistant		0	17	18	13
	% resistant		0	57	45	43

[1] I = Intestinal, C = Cecal
[2] S = Sensitive, R = Resistant

agents. Nine of the nitrofurazone-resistant strains were cross-resistant to sulfaquinoxaline and nitrophenide, while 3 were cross-resistant to sulfaquinoxaline and 4 to nitrophenide.

All of the 28 field strains studied were sensitive to nicarbazin. These strains included those which were sensitive to the individual anticoccidial agents as well as those possessing various cross-resistance patterns.

One of the strains of cecal coccidia (Maryland, No. 10) resistant to sulfaquinoxaline, nitrophenide and nitrofurazone was studied further in laboratory and pen-rearing experiments. The laboratory studies were performed by the standardized

TABLE 2.—*Comparative effect of certain anticoccidial agents on cecal Coccidia (Maryland Strain)*

Medication	% in Feed	% Mortality	Millions oocysts per chick
None	—	31 (14/45)*	38
Sulfaquinoxaline	0.05	26 (9/35)	28
"	0.1	31 (11/35)	21
"	0.2	20 (2/10)	23
Nitrophenide	0.05	12 (3/25)	12
Nitrofurazone	0.025	16 (4/25)	16
Sulfamerazine	0.1	60 (3/5)	33
"	0.2	20 (1/5)	32
"	0.4	0 (0/5)	5
Sulfaguanidine	1.0	0 (0/10)	1

* No. Dead/Total

prophylactic procedures. The data in Table 2 indicate that this strain was highly resistant to sulfaquinoxaline and at least moderately resistant to nitrophenide and nitrofurazone. This strain was also resistant to sulfamerazine but apparently sensitive to sulfaguanidine.

Further studies on nitrophenide and sulfaquinoxaline have shown (Table 3) that these compounds fail to control coccidiosis produced by graded inoculations of oocysts. However concurrent studies with nicarbazin have indicated that this anticoccidial agent in dosages of 0.01% was highly effective in controlling this strain of drug-resistant coccidia.

The pen-rearing experiment was conducted with New Hampshire chicks fed medicated rations from 1 day to 11 weeks of age. The chicks were exposed to coccidiosis when 3 weeks of age by administering the Maryland strain cecal oocysts (approximately 40,000/chick) in the drinking water. The results obtained from this experiment are shown in Table 4. These data have further confirmed that the recommended use concentrations of nitrofurazone, sulfaquinoxaline and nitrophenide are ineffective for this strain of coccidia. However, under these conditions, nicarbazin at concentrations of 0.01% and 0.02% in the feed has been highly to completely effective in controlling mortality produced by this virulent, drug-resistant strain of coccidia.

Laboratory strains.

Eimeria acervulina. The effect of sulfaquinoxaline on the development and infectivity of *E. acervulina* oocysts after exposure to sub-optimal concentrations

TABLE 3.—*Effect of Nicarbazin, Nitrophenide and Sulfaquinoxaline on graded inocula of resistant cecal Coccidia (Maryland Strain)*

Medication	% in Diet	Number of oocysts Inoculated: 6,250 % Mortality	6,250 Millions oocysts per chick	12,500 % Mortality	12,500 Millions oocysts per chick	25,000 % Mortality	25,000 Millions oocysts per chick	50,000 % Mortality	50,000 Millions oocysts per chick	100,000 % Mortality	100,000 Millions oocysts per chick
None	—	10	27	5	29	5	39	20	24	10	40
Nicarbazin	0.005	5	25	0	22	0	41	10	44	5	31
"	0.01	0	< 0.1	0	< 0.1	0	0.4	5	0.2	0	0.6
"	0.02	0	< 0.1	0	< 0.1	0	< 0.1	0	< 0.1	0	0.2
"	0.04	0	< 0.1	0	< 0.1	0	< 0.1	0	< 0.1	5	< 0.1
Nitrophenide	0.05	0	12	0	5	10	19	0	19	15	29
Sulfaquinoxaline	0.05	5	33	10	24	10	32	40	49	35	29

TABLE 4.—*The results of pen-rearing studies on medication of resistant cecal Coccidia (Maryland Strain)*

Medication	% in Feed	No. chicks started	Mean body weight at 11 weeks (lbs.)	Cecal coccidiosis Mortality (%)
None	—	310	2.87	63.2
Nicarbazin	0.005	155	2.88	58.1
"	0.01	155	2.85	25.8
"	0.02	155	2.60	0
"	0.04	155	2.52	0
Nitrofurazone	0.0066	155	2.64	61.3
Sulfaquinoxaline	0.015	155	2.94	67.1
Nitrophenide	0.025	155	2.86	60.6

of the drug for 18 serial passages during a period of 20 months has been determined. The data in Table 5 suggest that the 0.003% sulfaquinoxaline medication became less effective between the 6th to 10th passages. However, there was no further decrease in effectiveness detected during the next 12 passages. The only apparent effect of the 0.0015% sulfaquinoxaline medication was a decreased number of oocysts.

Although it was originally planned to run challenge titrations after 5 serial passages, this was not possible initially because of the inordinately low oocyst yield

TABLE 5.—*Effect of Sulfaquinoxaline on the production of* Eimeria acervulina *oocysts after serial passages in chicks during a period of 20 months*

Medication	% in Diet	Millions of oocysts per chick (Passage number)				
		0	1–5	6–10	11–15	16–18
None	—	28	—	—	—	—
Sulfaquinoxaline	0.0015	27	16	15	6	12
	Above controls	—	18	21	6	16
Sulfaquinoxaline	0.003	7	10	18	7	13
	Above controls	—	18	18	6	9
Sulfaquinoxaline	0.006	< 0.1	—	—	—	—

of the 5th passage. Consequently, the passages were continued and the first challenge titration was performed on the 10th passage material. The data in Table 6 reveal that resistance had developed in both the 0.0015% and 0.003% sulfaquinoxaline-exposed sub-strains. Although the resistance was possibly less for the 0.0015% sulfaquinoxaline-exposed sub-strain, both sub-strains were unquestionably less sensitive to sulfaquinoxaline now than initially. It was possible however, to inhibit oocyst production by increasing the inital dosage of sulfaquinoxaline 8 to 16 times. There was no evidence of further increase in resistance with 8 additional passages

TABLE 6.—*Effect of Sulfaquinoxaline on* Eimeria acervulina *after exposure to suboptimal concentrations of the drug for 18 serial passages*

Medication % Sulfaquinoxaline in the feed	Millions of oocysts per chick								
	10th Passage			16th Passage			18th Passage		
	(Previous exposure to Sulfaquinoxaline)								
	None	0.0015%	0.003%	None	0.0015%	0.003%	None	0.0015%	0.003%
None	15	19	11	5	2	2	16	10	32
0.0015	12	16	—	12	—	—	16	—	—
0.003	5	11	—	8	—	—	14	—	—
0.006	< 0.1	16	—	3	—	—	0.4	—	—
0.0125	—	19	12	—	3	3	0.4	14	33
0.025	—	5	15	—	18	7	—	28	36
0.05	—	1	7	—	7	15	—	4	5
0.1	—	< 0.1	0.1	—	< 0.1	0.1	—	0.3	0.1

and there was no indication that these sulfaquinoxaline-resistant sub-strains of *E. acervulina* were cross-resistant for any of the other anticoccidial agents used.

Eimeria tenella. Data have been obtained with 2 strains of cecal coccidia after 15 serial passages in the presence of sub-effective concentrations of sulfaquinoxaline. Concurrent studies were also conducted with nitrophenide and nitrofurazone for one strain and with nicarbazin, for another. The effect of sub-optimal amounts of these anticoccidial agents on the Pennsylvania and Oregon strains of *E. tenella* for 15 successive passages has been summarized in Table 7.

With the Pennsylvania strain, the results have indicated that more oocysts are produced by sub-strains exposed to sub-optimal dosages of sulfaquinoxaline after 5 serial passages than originally. However, there does not appear to be any further increase in numbers of oocysts after 10 additional passages. The results with the Oregon strain have also suggested that after 5 passages the 0.025% sulfaquinoxa-

TABLE 7.—*Effect of Sulfaquinoxaline, Nitrophenide, Nitrofurazone and Nicarbazin on the production of* Eimeria tenella *oocysts during serial passages*

Medication	% in Feed	Millions of oocysts per chick (Passage number)			
		0	1–5	6–10	11–15
None*	—	20	25	20	34
Sulfaquinoxaline*	0.0125	11	28	14	25
—	Above controls	—	25	22	34
Sulfaquinoxaline	0.025	7	14	13	14
—	Above controls	—	25	13	17
Nitrophenide*	0.0125	17	20	15	13
—	Above controls	—	23	22	21
Nitrophenide	0.025	2	4	0.8	0.5
—	Above controls	—	26	19	19
Nitrofurazone*	0.003	4	15	14	21
—	Above controls	—	23	29	29
Nitrofurazone	0.006	3	6	6	8
—	Above controls	—	20	17	21
None**	—	22	10	12	11
Nicarbazin**	0.005	18	8	8	9
—	Above controls	—	6	10	8
Nicarbazin	0.01	0.4	2	3	3
—	Above controls	—	10	17	11
Sulfaquinoxaline**	0.0125	18	4	10	12
—	Above controls	—	7	14	11
Sulfaquinoxaline	0.025	1	0.6	5	6
—	Above controls	—	3	7	6

* Pennsylvania strain
** Oregon strain

line sub-strain has become slightly less responsive to the compound. A similar change occurred with a 0.0125% sulfaquinoxaline sub-strain after 10 passages. Under these same experimental conditions, the Pennsylvania strain of *E. tenella* has not changed in responsiveness to 0.0125% nitrophenide, while the sub-strain exposed to 0.025% nitrophenide has, if anything, become more sensitive. Similar studies with nitrofurazone suggested that the sub-strain exposed to 0.003% of the drug produces more oocysts after 5 to 15 passages than at first. However, the 0.006% sub-strain has been quite consistent in the numbers of oocysts produced.

The results of challenge titrations with the several medication-exposed sub-strains are shown in Table 8. These data indicate that the Pennsylvania sub-strain exposed to 0.0125% sulfaquinoxaline has not changed in responsiveness, while the 0.025% sulfaquinoxaline strain has become less sensitive to sulfaquinoxaline after 5 passages. With 10 additional passages the 0.0125% sulfaquinoxaline-exposed strain appears as resistant as the 0.025% sub-strain, which has shown no further increase, however. A similar response appears to have occurred with the Oregon

strain of *E. tenella*. It is concluded that both of these strains have become less sensitive to sulfaquinoxaline after 5 to 10 passages in the presence of sub-optimal dosages.

The data, also in Table 8, indicate that, under these experimental conditions, the nitrophenide and nitrofurazone exposed sub-strains of *E. tenella* (Pennsylvania strain) have not changed detectably in sensitivity to these agents after 15 serial passages in the presence of sub-optimal concentrations of the drugs.

Similar studies have also been made with the Oregon strain of *E. tenella* exposed to sub-optimal concentrations of nicarbazin for 15 serial passages. The effect of nicarbazin on the production of oocysts after 5, 10 and 15 passages and the results

TABLE 8.—*Effect of Sulfaquinoxaline, Nitrophenide, Nitrofurazone and Nicarbazin on production of* Eimeria tenella *oocysts after exposure to suboptimal concentrations of the drugs for 15 serial passages*

Medication	% in Feed	Millions of oocysts per chick								
		5th Passage			10th Passage			15th Passage		
		(Previous exposure to Sulfaquinoxaline)								
		None	0.0125%	0.025%	None	0.0125%	0.025%	None	0.0125%	0.025%
None*	—	37	35	37	16	44	13	34	19	21
Sulfaquinoxaline	0.05	0.7	0.5	10	1	2	7	0.6	7	6
Sulfaquinoxaline	0.1	—	< 0.1	< 0.1	—	< 0.1	0.3	—	0.3	2
None**	—	21	12	10	16	24	19			
Sulfaquinoxaline	0.05	0.1	< 0.1	2	< 0.1	1	2			
		(Previous exposure to Nitrophenide)								
		None	0.0125%	0.05%	None	0.0125%	0.025%	None	0.0125%	0.025%
None*	—	37	39	30	16	26	23	34	18	22
Nitrophenide	0.05	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Nitrophenide	0.1	—	< 0.1	< 0.1	—	< 0.1	< 0.1	—	< 0.1	< 0.1
		(Previous exposure to Nitrofurazone)								
		None	0.003%	0.006%	None	0.003%	0.006%	None	0.003%	0.006%
None*	—	37	38	27	16	23	27	34	20	19
Nitrofurazone	0.0125	—	2.2	1.5	—	< 0.1	0.3	—	0.2	0.5
Nitrofurazone	0.025	1.5	< 0.1	0.5	< 0.1	< 0.1	< 0.1	0.2	< 0.1	< 0.1
		(Previous exposure to Nicarbazin)								
		None	0.005%	0.01%	None	0.005%	0.01%	None	0.005%	0.01%
None**	—	21	23	17	16	11	16			
Nicarbazin	0.02	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1			

* Pennsylvania strain.
** Oregon strain.

of challenge titrations are also shown in Table 8. The data have indicated that these sub-strains are equally as responsive to nicarbazin after 15 passages as originally.

DISCUSSION

The use of chemcials for controlling coccidiosis has been demonstrated to be a successful and practical way to minimize the economic losses which may occur from this major disease hazard in poultry. Because of many variables in this disease, such as species and virulence of coccidia, host susceptibility, seasonal and climatic conditions, and management and sanitation practices, it has not always been possible to use the dosages of anticoccidial drugs which might be most effective. Actually, the practice has developed of giving the anticoccidial medication

in the minimal dosage which will control the most severe symptoms of the disease and yet permit the development of immunity. Consequently, in some cases the infections may be treated with sub-optimal amounts of the anticoccidial agents. Although the disease outbreak is controlled, many oocysts are discharged and these may re-infect birds in the same or subsequent flocks. When this procedure is repeated 3 or 4 times per year, and for several successive years, there appears to be an excellent opportunity for coccidia to develop an increasing tolerance to the chemicals.

Although Harwood and Stunz (1953) were unable to find any indication of drug-resistance in 2 field strains of coccidia, the results of our studies on a relatively small number of field strains of coccidia have indicated that resistance has been acquired for 3 of the most widely used anticoccidial drugs. There was a higher percentage of strains resistant to nitrofurazone than to sulfaquinoxaline or nitrophenide, but the number of observations is insufficient for making definite conclusions regarding the relative rate at which resistance is acquired. In general, however, considering the many places where anticoccidial medication has been employed, it does not appear probable that development of resistance in coccidia has occurred frequently.

All strains studied have been sensitive to nicarbazin. There does, however, seem to be a tendency for cross-resistance to prevail for the other agents which have been used in the field. An apparently similar relationship has been shown by Bishop and McConnachie (1950, 1952) for avian malaria. Strains of *Plasmodium gallinaceum* made resistant to sulfadiazine or pamaquin were also cross-resistant to certain antimalarial agents but not to others. This also appears consistent with what has been observed for trypanosomes (Work and Work [1948]). This may suggest that in a given population of coccidia, plasmodia, or trypanosomes there are spontaneously occurring variants or mutants for which any one of several drugs might have the same selective action. Whether acquired drug resistance in coccidia is an example of selective drug action between different strains of the same parasite, we are unable to conclude, however, from the relatively meager data available.

In our studies with laboratory strains we have obtained evidence that resistance may be induced in coccidia exposed to sulfaquinoxaline. The data we have presented suggest that *Eimeria acervulina* may acquire significant tolerance to sulfaquinoxaline after 10 passages in the presence of a therapeutically ineffective concentration of the drug. However, the data for 2 strains of *Eimeria tenella* suggest that only a detectable tolerance may be induced in this species after exposure to sulfaquinoxaline for 5 to 10 passages. There is no evidence of further increased tolerance in either of these species of coccidia with 5 to 8 additional transfers. Neither of these laboratory-induced sulfaquinoxaline-resistant strains appears to have cross-resistance for the other anticoccidial agents studied.

Similar laboratory studies with nicarbazin, nitrophenide and nitrofurazone have not revealed any evidence of induced resistance in one strain of *E. tenella* exposed to sub-effective concentrations of these drugs for 15 passages. However, on the basis of evidence of nitrophenide and nitrofurazone resistance in field strains of coccidia, it appears reasonable to assume that induced resistance to these drugs

might have been demonstrated if additional strains of coccidia or longer periods of exposure had been used.

SUMMARY

1. Studies on 40 field strains of allegedly resistant coccidia have revealed that 43 percent were sensitive to one or more of the drugs studied but that 43 percent were resistant to nitrophenide, 45 percent to sulfaquinoxaline and 57 percent to nitrofurazone. Some of the strains were resistant to all of these anticoccidial drugs, while others were resistant to 2 of the compounds. In some strains specific resistance was observed, but cross-resistance occurred more frequently than specific resistance. However, in view of the continued generally satisfactory use of anticoccidial medication, it does not appear probable that development of resistance in coccidia has become an important practical problem as yet.

2. Experimentally induced resistance has been demonstrated for 1 strain of *Eimeria acervulina* and 2 strains of *Eimeria tenella* after exposure to sub-optimal dosages of sulfaquinoxaline for 15 serial passages.

3. There was no evidence that 1 strain of cecal coccidia exposed to sub-optimal dosages of nitrophenide, nitrofurazone or nicarbazin for 15 series passages has become less sensitive to these anticoccidial agents.

4. Nicarbazin effectively inhibited all strains of coccidia whether sensitive or resistant to other agents. Coccidia which were markedly resistant to sulfaquinoxaline, nitrophenide, nitrofurazone, and sulfamerazine were completely susceptible to nicarbazin.

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REFERENCES

BISHOP, A. AND MCCONNACHIE, E. W. 1950 Sulfadiazine-resistance in *Plasmodium gallinaceum* and its relation to other antimalarial compounds. Parasitology **40:** 163–174.

———1952 Pamaquin-resistance in a strain of *Plasmodium gallinaceum* and it relationship to other antimalarial drugs. Parasitology **42:** 57–64.

CUCKLER, A. C., MALANGA, C. M., BASSO, A. J. AND O'NEILL, R. C. 1955 The antiparasitic activity of some molecular complexes of substituted carbanilides. *In preparation.*

EHRLICH, P. 1907 Chemotherapeutische Trypanosomen-Studien. Berl. klin. Wochenschr. **44:** 223–236, 280–283, 310–344, 349–350.

HARWOOD, P. D. AND STUNZ, D. I. 1953 A search for drug-fast strains of *Eimeria tenella.* J. Parasit. **39:** 268–271.

WORK, T. S. AND WORK, E. 1948 The basis of chemotherapy. Interscience Publishers, Inc. New York. xviii + 435 pages.

TWO NEW SPECIES OF TICKS FROM NORTH BORNEO (ACARINA: IXODIDAE)[1]

GLEN M. KOHLS

Examination of the extensive tick collections made by the joint U. S. Army–British Colonial Office Medical Research Unit in North Borneo during recent investigations on arthropod vectors and rodent reservoirs of disease has resulted in the discovery of the two new species here described. I am indebted to Lt. Colonel Robert Traub, Medical Service Corps, U. S. Army Medical Service Graduate School, for submitting the collections to me for study.

Ixodes audyi n. sp.
(Fig. 1)

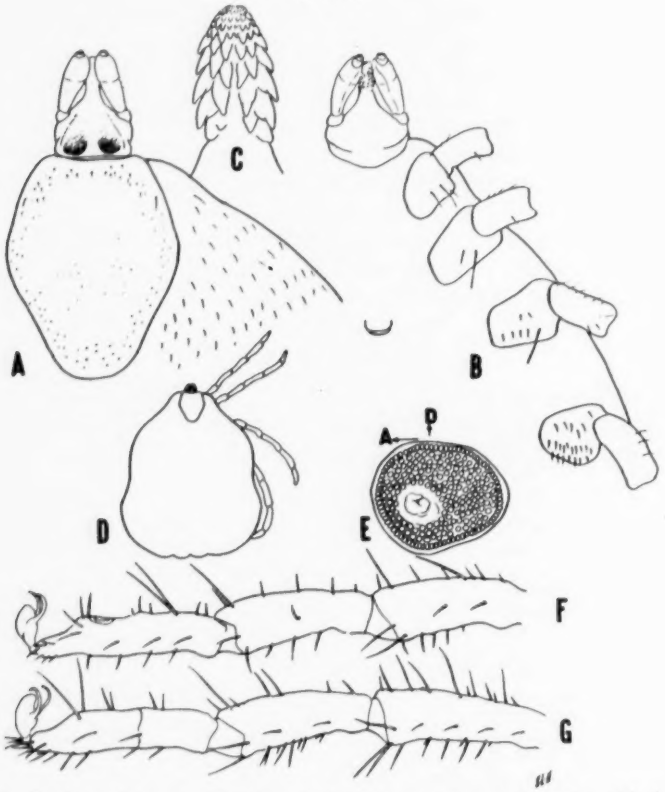

FIG. 1. *Ixodes audyi* n. sp. Female. A. Capitulum, scutum, and portion of body showing hairs. B. Capitulum and coxae, venter. C. Hypostome. D. Engorged female. E. Spiracular plate. F. Tarsus and metatarsus, leg I. G. Tarsus and metatarsus, leg IV.

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[1] From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Microbiological Institute, Rocky Mountain Laboratory, Hamilton, Montana.

Described from a single well-engorged female, the holotype, off *Rattus infraluteus*, Mari Parei, 5100 feet elevation, Mt. Kinabalu, August 28, 1953 (R. Traub). Deposited in the Rocky Mountain Laboratory Collection; RML No. 32822.

Body. Length, excluding the capitulum, 4.5[2]; width 3.5. Shape as illustrated. Dorsal and ventral surfaces with numerous hairs but these are absent in the anal and genital grooves.

Capitulum. Length, 0.54; width of basis, 0.43. Posterior margin of basis salient and sinuous. Cornua absent. Porose areas large, subcircular, and separated by less than half the diameter of one. Palpi moderate in length, broad and rounded apically; articulation between segments 2 and 3 only faintly visible. Segment 2 only slightly longer than 3; combined length about 0.30. Ventrally the basis is wider in front and broadly rounded behind. Auriculae faintly indicated as mild lateral ridges. Transverse sutural line absent.

Hypostome. Length about 0.18. Shape and dentition as figured.

Scutum. Shape as figured. Length, 1.14; width, 0.94; widest at about the middle. Scapulae very short and blunt. Lateral carinae and cervical grooves absent. Punctations about equal in size, scattered, somewhat more numerous in the depressed anterolateral areas where the surface is somewhat irregular. Some of the punctations with short fine hairs.

Legs. Moderate in length. All coxae without spurs, posterior margins a little salient. Coxa IV with about 17 short fine hairs arranged in two transverse rows near the hind margin, plus about six similar hairs situated anteriorly; other coxae with a few scattered hairs. All tarsi gradually narrowed distally; tarsus I with a mild subterminal hump. Length of tarsus I, 0.78; metatarsus, 0.65. Length of tarsus IV, 0.77; metatarsus, 0.65.

Spiracular plate. Suboval; greatest dimension about 0.31.

Genital aperture. Situated between coxae III.

Male and immature stages unknown.

The absence of coxal spurs and the presence of numerous hairs on the fourth coxa will readily distinguish this species from the females of the few other species of this genus known to occur in the Malaysian region.

This new species is named for Dr. J. R. Audy, Director of the Colonial Office Medical Research Unit (now the Division of Virus Research and Medical Zoology), Institute for Medical Research, Kuala Lumpur, Malay, who has contributed much to our knowledge of scrub typhus and other arthropod-borne diseases.

Haemaphysalis traubi n. sp.
(Fig. 2)

Described from three males found on the barking deer, *Muntiacus muntjak*, at Tenompak, 5500 feet elevation, Mt. Kinabalu, North Borneo, July 7, 1951 (D. Johnson and P. A. Wah), RML No. 30189. Holotype and paratype in the Rocky Mountain Laboratory; one paratype in the British Museum (Natural History).

Body. Length from tips of palpi to posterior margin of body (three specimens) 2.35, 2.35, 2.28; width, 1.39, 1.44, 1.39. Suboval, widest slightly anterior to the spiracular plates.

Capitulum. Length from tips of palpi to tips of cornua from 0.48 to 0.51. Length of basis including cornua about 0.24; width about 0.34. Surface of basis with a few scattered shallow punctations; lateral margins slightly convex. Posterior margin between the cornua evenly curved. Cornua strong, about 0.10 in length, pointed. Palpal segment 2 only slightly salient laterally and about equal to segment 3 in length. No dorsal spur on segment 3 but posterior margin is somewhat salient. In ventral view the surface of the basis is mildly convex, the posterior margin nearly straight. Palpal segment 2 without a spur, inner margin with 4–5 hairs directed toward the base of the hypostome. Palpal segment 3 with a long, sharp, retrograde spur.

Hypostome. Shape as figured. Dentition 4/4 with 6 or 7 denticles in each file. Denticles in the median files only slightly smaller than those in the laterals. Length of toothed portion about 0.16.

Scutum. Length from tips of scapulae to posterior margin 1.92 to 2.00. Scapulae bluntly rounded. Lateral grooves absent. Cervical grooves merely as depressions to receive the cornua. Punctations numerous, but small and inconspicuous.

Legs. Coxal spurs small but definite, decreasing progressively in size from I to IV. Trochanters each with a ventral spur somewhat larger than the coxal spur of the same leg.

[2] All measurements in millimeters.

Trochanter of leg I with a well developed dorsal spur as figured. Length of tarsus I, 0.52; metatarsus, 0.29; length of tarsus IV, 0.50; metatarsus, 0.35. Tarsi without ventral spurs.

Spiracular plate. Shape as figured. Greatest dimension about 0.28.

Genital aperture. Situated between coxae II.

This new species bears a superficial resemblance to *H. birmaniae* Supino to which it runs in Nuttall and Warburton's (1915) key. There are three males from Ceylon in the Rocky Mountain Laboratory collection determined as *H. birmaniae* by the above authors. *H. traubi* is readily distinguishable from this species by the much longer spur on palpal segment 3 ventrally and the much longer and more strongly developed cornua. The dorsal trochantal spur of coxa I is also much more pointed than in *H. birmaniae*, in which it is blade-like as in most other species of the genus. Moreover, the cervical grooves are in the form of excavations

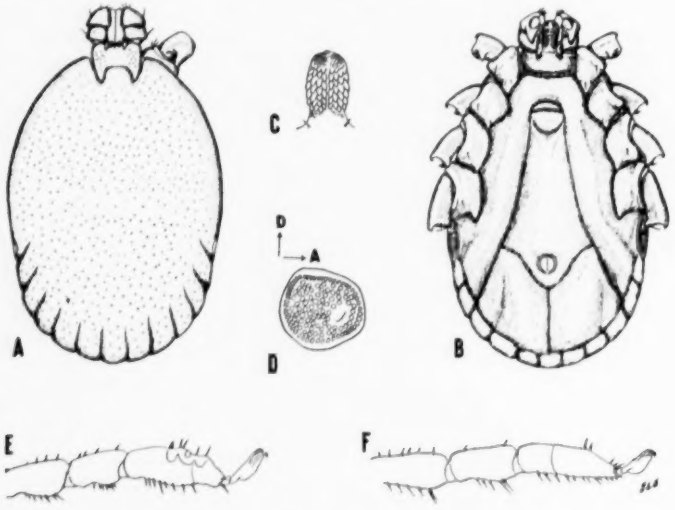

FIG. 2. *Haemaphysalis traubi* n. sp. Male. A. Capitulum and scutum, dorsum. B. Capitulum, coxae and body, venter. C. Hypostome. D. Spiracular plate (A = anterior; D = dorsal). E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV.

into which the cornua fit when the capitulum is elevated, rather than small oval pits situated some distance behind the anterior border as in *H. birmaniae*. Except for the long retrograde ventral spur on palpal segment 3, the above mentioned characters will also distinguish the new species from *H. palawanensis* Kohls, 1950, and *H. rusae* Kohls, 1950, in both of which the lateral grooves are also absent. In the former the palpi are much more salient basolaterally than in *H. traubi* and the denticles of the hypostome are arranged 5/5. Several characters including those of the hypostome and legs as well as the absence of a retrograde spur on palpal segment 2 ventrally will serve to distinguish the new species from *H. rusae*.

It is a pleasure to name this new species for Lt. Colonel Robert Traub in recognition of his distinguished contributions to studies of ectoparasites in many parts of the world.

SUMMARY

Ixodes audyi n. sp. and *Haemaphysalis traubi* n. sp. are described from Mt. Kinabalu, North Borneo. Hosts were *Rattus infraluteus* and *Muntiacus muntjak*, respectively.

REFERENCES

KOHLS, G. M. 1950 Ticks (Ixodoidea) of the Philippines. National Instit. Health Bull. No. 192, U. S. Pub. Health Serv. 28 pp.

NUTTALL, G. H. F. AND WARBURTON, C. 1915 The genus *Haemaphysalis*, Pt. III, Ticks: A monograph of the Ixodoidea, by Nuttall, Warburton, Cooper, and Robinson. Cambridge University Press. pp. i–xiii and 349–550, pls. VIII–XIII.

EXPERIMENTAL TESTING OF THE IMMUNITY OF NEGROES TO *PLASMODIUM VIVAX*[1]

MARTIN D. YOUNG, DON E. EYLES,[2] ROBERT W. BURGESS, AND GEOFFREY M. JEFFERY

One of the first observations made after this laboratory was established in 1931 was that American Negroes are relatively insusceptible to *Plasmodium vivax*, (Mayne, 1932). Since then much additional evidence of a similar nature has been obtained in this laboratory and by others (Boyd and Stratman-Thomas, 1933) for native strains of *P. vivax*.

During World War II, it was shown that introduced foreign strains of *vivax* from many parts of the world exhibited a similar inability to infect Negroes (Young, Ellis, and Stubbs, 1946; Becker, Kaplan, *et al*, 1946). However, Butler and Sapero (1947) reporting on troops based on a South Pacific island stated that the susceptibility of the American Negro to Pacific *vivax* malaria does not differ from that of the white American. This report differs so widely from the common experience that we have critically examined the data gathered in this laboratory for a period exceeding 20 years to determine if any of the strains tested were uniformly infective to Negroes. Furthermore, experiments were set up to discover if the immunity of Negroes could be overcome by massive inoculations of malaria parasites which, we thought, might explain the difference between Butler and Sapero and the other workers. Our results are given in the present report.

MATERIALS AND METHODS

Neurosyphilitic patients requiring malaria therapy were inoculated either by the bites of infected mosquitoes or by the injection of infected blood. Most of these patients were civilians but a few were military personnel in service hospitals.

The domestic malarias tested originated principally from patients in South Carolina but some strains were obtained from hospitals in other parts of the United States employing induced malaria. The principal domestic strain tested was the St. Elizabeth. The foreign strains of malaria were obtained from military personnel

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returning from foreign duty during the World War II and Korean war periods. Included was the Chesson (New Guinea) strain which has been used widely for drug testing.

The mosquitoes used as vectors were principally *Anopheles quadrimaculatus* and *A. freeborni*, both good transmitters of domestic and foreign malarias (Young, Eyles, and Burgess, 1948). The bites of ten well-infected mosquitoes have been set as the desired standard inoculum but, as this exact number was not always possible, in this report we list "normal" inoculations as being from one to 40 mosquito bites. Over 40 mosquito bites are considered as "massive" inoculations and undoubtedly represent many times the infective dose of sporozoites ordinarily received in nature.

The experience over a number of years at this hospital shows that the routine inoculations to white neurosyphilitics contained an average of about 35,000,000 parasites. For this report dosages up to 100,000,000 parasites are listed as normal; those above 100,000,000 are considered massive.

OBSERVATIONS

The results of inoculating white and Negro persons with normal amounts of *vivax* malaria are summarized in Table 1. Infections resulted in 23.1 percent of the

TABLE 1.—*Normal inoculations of* P. vivax *by mosquito bites or infected blood to white and Negro patients. First inoculations only*

Origins of strains	Mode of inoculation	NEGROES			WHITES		
		Number of patients	Number infected	Percent infected	Number of patients	Number infected	Percent infected
Pacific (includes Chesson)	Mosquito	33	13	39.4	199	189	95.0
	Blood	5	1	20.0	38	38	100.0
		38	14	36.8	237	227	95.8
Korean	Mosquito	6	0	0.0	10	8	80.0
	Blood	2	1	50.0	3	3	100.0
		8	1	12.5	13	11	84.6
Mediterranean	Mosquito	20	6	30.0	33	31	93.9
	Blood	1	0	0.0			
		21	6	28.6	33	31	93.9
Domestic (includes St. Elizabeth)	Mosquito	13	1	7.7	120	115	95.8
	Blood	24	2	8.3	126	125	99.2
		37	3	8.1	246	240	97.6
GRAND TOTALS		104	24	23.1	529	509	96.2

Negroes and in 96.2 percent of the white people. Under the conditions of normal inoculations, it is obvious that Negroes are far more refractory to *P. vivax* than are white persons.

Experiments involving massive inoculations were done and the results are shown in Table 2. The massive inoculations did not increase the proportion of Negroes developing *vivax* malaria over that resulting from normal inoculations. In fact, the percentages were lower in the massive group.

An examination of some of the experiments give interesting sidelights. A group of three men were exposed to the same lot of infected mosquitoes for bites; one man was exposed every day for 14 days receiving a total of 161 bites, the second man receiving 56 bites in four days, and the third (control) receiving 16 bites on one day

only. The latter, who had received only one-tenth and one-third, respectively, of the number of bites as had his two partners, was the only one of the three who developed malaria.

Another group of three patients received in a single day from the same mosquitoes the following numbers of bites: A. H.—225 bites, M. B.—206 bites, and F. G.—214 bites. Of these, only F. G. developed an infection.

The above instances add to the evidence that the amount of inoculum is not the primary factor in determining infectivity.

To determine if a *vivax* infection once established in a Negro is altered so that it could then be more easily transmitted to others of the same race, two patients were inoculated with blood from F. G., receiving 108 and 235 million parasites respectively. Neither of these recipients showed parasites subsequent to the massive inoculation, indicating that the infected donor had not modified the *vivax* strain so that it would more easily parasitize Negroes.

TABLE 2.—*Massive inoculations of Negroes with* P. vivax *by mosquito bites and inoculation of infected blood*

Parasite strain	No. days inoculated	Mosquito bites			Patients		
		Total number bites			Number exposed	Number infected	Percent infected
		Range	Average	Median			
Chesson	1	41–225	97.8	91	12	1	8.3
Chesson	4	56			1	0	0.0
Chesson	14	161			1	0	0.0
St. Elizabeth	14	165–167	166	166	2	0	0.0
Sub-Totals					16	1	6.3
	Inoculation of Infected Blood: Parasites 10^6						
Chesson		108–1177	302.3	219	9	1	11.1
St. Elizabeth		162	162		2	0	0.0
Sub-Totals					11	1	9.1
GRAND TOTALS					27	2	7.4

The largest inoculation in the experiments contained $1{,}177 \times 10^6$ parasites. The patient receiving this inoculum showed no parasites in the blood either the following day or subsequently. By contrast, an inoculation one-tenth this size often produces a parasitemia in white patients that is patent immediately after inoculation and is present continuously throughout the primary attack.

DISCUSSION

Since 1931, many domestic strains of *vivax* malaria have been given to Negroes. Our experience has confirmed and extended that of others who use malaria in the treatment of neurosyphilis in that most Negroes are refractory to this species of malaria.

During the recent war years, our laboratories have tested in Negroes many strains of *vivax* malaria from foreign countries, namely, Tunisia, Sicily, Italy, Korea, and the Southwest Pacific. These foreign strains also were unable to infect Negroes consistently. The Negro seems to have a general resistance to strains from all areas rather than to specific strains.

In previously reported controlled experiments (Young, *et al*, 1946), it was shown that Negroes from non-malarious areas of the United States also showed resistance

to *vivax* infection. This indicates that the immunity is natural rather than acquired.

The above refractoriness was not overcome by the use of massive dosages of parasites. In fact there was no evidence that increasing the dosage has any effect on increasing the rate of infection. Actually the highest dosage did not produce as many infections as lower dosages.

An exception to the general experience that Negroes show an immunity to *P. vivax* is that of Butler and Sapero (1947) who concluded that the American Negro lacks racial tolerance to the strains encountered in the Pacific and that his susceptibility does not differ from that of the American white person. Several possible explanations of their finding can be presented: 1. There is a strain of *vivax* malaria, not yet among the many tested experimentally, which infects Negroes generally. 2. The troops were under unrecognized conditions which made them unusually susceptible to malaria. One obvious factor, an increase in exposure to the parasite, has been tested experimentally and found not to be important.

The verification of either of the above possibilities would add to the biological knowledge of the parasite and would be of considerable interest to malariologists.

Summary and Conclusions

Negroes in general exhibited a refractoriness to many domestic and foreign strains of *Plasmodium vivax* under the conditions in which white persons were susceptible.

Massive dosages of parasites, given by blood inoculation or by the bites of mosquitoes, failed to overcome the immunity shown by most Negroes.

A *vivax* infection in one Negro was not infective to Negro recipients inoculated therefrom.

References

Becker, F. T., Kaplan, L. I., Read, H. S., and Boyd, M. F. 1946 Variations in susceptibility to therapeutic malaria. Am. J. Med. Sci. **211**: 680–685.

Boyd, M. F., and Stratman-Thomas, W. K. 1933 Studies on benign tertian malaria. 4. On the refractoriness of Negroes to inoculation with *Plasmodium vivax*. Am. J. Hyg. **18**: 485–489.

Butler, F. A., and Sapero, J. J. 1947 Pacific *vivax* malaria in the American Negro. Am. J. Trop. Med. **27**: 111–115.

Mayne, B. 1932 Note on experimental infection of *Anopheles punctipennis* with quartan malaria. Pub. Health Rep. **47**: 1771–1774.

Young, M. D., Ellis, J. M., and Stubbs, T. H. 1946 Studies on imported malarias: 5. Transmission of foreign *Plasmodium vivax* by *Anopheles quadrimaculatus*. Am. J. Trop. Med. **26**: 477–482.

Young, M. D., Eyles, D. E., and Burgess, R. W. 1948 Studies on imported malarias: 10. An evaluation of the foreign malarias introduced into the United States by returning troops. J. Nat. Mal. Soc. **7**: 171–185.

ZOOLOGICAL NOMENCLATORIAL NOTES

The following Opinions of the International Commission on Zoological Nomenclature, recently published, are of special interest to parasitologists:

OPINION 283. Validation, under the Plenary Powers, of the generic and specific names commonly used for the Malignant Tertian Malaria Parasite and the Quartan Malaria Parasite respectively. *Opinions and Declarations Rendered by the International Commission on Zoological Nomenclature*, London, Vol. 7, Part 1, Pp. 1–226. (Issued 28 Dec. 1954).

OPINION 312. Validation, under the Plenary Powers, of the name *Amoeba coli* as from Grassi, 1879, to be the name for the large non-dysenteric amoeba of Man and the designation of that species to be the type species of the genus *Entamoeba* Casagrandi & Barbagallo, 1895, and designation under the same powers of the name *Entamoeba histolytica* Schaudinn, 1903, to be the name for the dysenteric amoeba of Man (Class RHIZOPODA) (Opinion substituted for Opinion 99). Ibid. Vol. 9, Part 1, Pp. 1–60. (Issued 17 Dec. 1954).

Opinion 283 includes the placing in the "Official List of Generic Names in Zoology" of

a) *Plasmodium* Marchiafava & Celli, 1885 (gender: neuter) of which the type species by designation under the plenary power is *Haemamoeba malariae* Feletti and Grassi, 1889 (the quartan malaria parasite); and

b) *Laverania* Feletti and Grassi, 1889 (gender: feminine) of which the type species by designation under the plenary powers is *Haematozoon falciparum* Welch, 1897 (the malignant tertian malaria parasite) as the generic name to be used by authors who consider the malignant tertian (i.e. aestivo-autumnal) to be generically distinct from the quartan malaria parasite.

As the title of Opinion 312 indicates, Grassi's 1879 species *coli* is now the officially designated type species for the genus *Entamoeba* Casagrandi and Barbagallo, 1895, and *Entamoeba coli* and *E. histolytica* are approved names for, respectively, the large non-dysenteric and the dysenteric amoebae of man. The opinion also places on the "Official List of Generic Names in Zoology"

Poneramoeba Lühe, 1909, for use by those who consider *Entamoeba histolytica* to be generically distinct from the large non-dysenteric amoeba of man.

The above constitutes a necessarily brief and incomplete statement of the rulings taken by the Commission, which need to be read in their entirety (Op. 283, pp. 3–12; Op. 312, pp. 3–6). The extensive historical analyses upon which the decisions in both opinions have been based include significant new materials hitherto overlooked, and become valuable sourcebooks in protozoology on that account alone.

To students of taxonomic aspects it will be further of interest that since January, 1954 the Commission has made great progress in clearing its desk through the publication, under Secretary Henming, of 16 Declarations (Nos. 1–9, 13–19) and 134 Opinions (Nos. 195–329). Included are those relating to *Necator* (Op. 201), *Schistosoma* (Op. 226), *Fasciola ovata* (Op. 227), *Piroplasma annulatum* (Op. 266), *Taenia* (Op. 272), and *Leptopsylla* (Op. 285). Opinions in press include those on *Stephanurus* (Op. 340), *Anguina* (Op. 341), and *Lipeurus* (Op. 342).

The effort is made to have the publications of the Commission (which has no financial endowment) pay their own way. They are sold on behalf of the I.C.Z.N. by the International Trust for Zoological Nomenclature, 41, Queen's Gate, London, S. W. 7, and may be individually purchased.—NORMAN R. STOLL, *The Rockefeller Institute, New York.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirtieth Annual Meeting
Preliminary Announcement

Wednesday, Thursday, and Friday, December 28 to 30, 1955
Atlanta, Georgia

This is to remind all members that titles and abstracts of all papers must be in the hands of the Secretary not later than September 15, 1955, in order to be included in the printed program. All members are urged to present their papers by demonstration where at all possible to do so, and thus relieve the other sessions of the overcrowding of the past few years. Emory University offers ample facilities for such demonstrations.

Council Members should note that the Council Meeting and Dinner will be held Tuesday Evening, December 27, 1955, to fit in with the A.A.A.S. program.

Housing will be reserved through the A.A.A.S. Housing Bureau. Watch Science for housing information.

Individual notices will be sent out as soon as details for meeting rooms have been settled, probably early in June.

A.C. Walton, *Secretary*

ERRATA

Page 43, the first line under "Discussion" should read "The germinal zone of the ovary of *Trichinella spiralis* is like that of Trichuris," etc.

Page 195, first line of data in table II, second column, should read "5 females, 0 males."

RESEARCH NOTES

RE-DISCOVERY OF THE PARASITIC CIRRIPEDE, *MYCETOMORPHA VANCOUVERENSIS* POTTS, IN BRITISH COLUMBIA WATERS

An unusual parasite of the shrimp *Crago communis* (Rathbun) was collected by F. A. Potts near Departure Bay, British Columbia, in August, 1911. Subsequently, on the basis of this specimen, Potts (1912, Zool. Jahrb. **33**: 575–594), published the diagnosis of a new genus *Mycetomorpha* and fully described the new species *M. vancouverensis*.

Four additional specimens of *M. vancouverensis* have been collected; off the west coast of Vancouver Island, June 5, 1934 by E. G. Hart, at two localities in the Strait of Georgia, May 3, 1949 and April 21, 1953 by the writer, and in the Strait of Juan de Fuca, August 8, 1954 by G. Dubokovic. The hosts were *Crago communis*. The parasites were not sectioned, but differed very little in external anatomy and size from the description of Pott's specimen.—T. H. Butler, *Pacific Biological Station, Nanaimo, B. C., Canada.*

ON THE EARLY DEVELOPMENT OF *PORROCAECUM DECIPIENS*

The eggs of a marine ascarid, *Porrocaecum decipiens* (Krabbe), are laid in the stomachs of seals (Scott, 1953, J. Fish. Res. Bd. Canada, **10**: 539–547) and pass out to the sea in the feces of seals. As part of an investigation on the life cycle of *P. decipiens*, some simple experiments were carried out to determine if the eggs of this parasite would hatch in sea water. The work was sponsored by the Fisheries Research Board of Canada and done in August, 1949, at the Atlantic Biological Station, St. Andrews, New Brunswick, under the direction of Dr. W. R. Martin.

Three gravid female worms were taken from the stomach of an experimentally-infected harbor seal, *Phoca vitulina* (Linnaeus). The uteri of the worms were removed and about 100 eggs were taken from the vaginal end of each uterus. The three lots of eggs were thoroughly mixed together, and from this mixed lot about 50 eggs were placed in each of three Syracuse watch-glasses. Sterile conditions were not provided.

Each lot of eggs was kept at a different temperature: one in darkness at 2° to 4° C., and a second in darkness at 13° to 14° C.; these temperatures fall within the range of temperatures to which the eggs of *P. decipiens* might ordinarily be exposed in the sea. The third lot was kept on the author's desk and was exposed to normal daily light changes and to a wide range of temperatures (about 17° to 25° C.) which would not be commonly encountered by the eggs of *P. decipiens* under natural conditions.

The eggs adhered firmly to the bottom of the watch glasses; this permitted daily changes of sea water without any loss or undue disturbance of the eggs. Examinations of the eggs were made at intervals of 24 hours or less.

The eggs had begun to develop when they were removed from the uteri. Although a few eggs in the 16-cell stage were seen, most were in the 4-cell stage. At this stage the eggs were almost spherical; the average dimensions of 10 eggs were 52 microns in length by 45 microns in width.

Development at 2° to 4° C. was very slow; indeed, it was so slow that only the absence of degenerative changes indicated that the eggs were still alive. The morula stage was first observed by the end of four days and the gastrula stage was not reached until 18 days had elapsed. By the end of the experiments after 27 days no movements of larvae within the eggs had been detected.

At 13° to 14° C. development was much more rapid than that observed at 2° to 4° C. The morula stage was present after three days and gastrulae were noted by the end of the fifth day. Movement of some embryos was observed after seven days. Some eggs hatched after 13 days; the remainder of this lot had hatched by the end of the 14th day. After hatching the larvae remained close to the empty eggs and became fixed by their posterior ends to the bottom of the watch glass. The larvae were very active, wriggling and gyrating about a vertical plane. Once they had become attached to the bottom, they remained in this position and did not move horizontally along the bottom of the dish. Ten larvae averaged about 200 microns in length at this time. Little anatomical detail could be seen: a small boring tooth was visible; anteriorly the esophagus and ventriculus were differentiated, but the rest of the digestive system was not clearly defined. The newly-hatched larvae were encased in loose-fitting sheaths, which probably were the moulted cuticles of the first-stage larvae. Although the larvae remained fully active until the end of the experiments, 13 days later, no larva was able to get rid of the old cuticle.

The most rapid development occurred at 17° to 25° C. The morula stage was reached after two days, gastrulae were observed after three days, and movement of some embryos

was noticed after four days. Hatching began for some individuals as early as the 8th day and was completed for the whole group by the end of the 9th day. Following hatching, all worms died, apparently within a few hours. Although the actual process of hatching was not observed in this group, many empty egg-shells were seen closely associated with amorphous masses of dark material which were presumably the remains of dead larvae. In a few cases amorphous material was also seen extending from within the egg to the outside as though the larva had died during hatching. The cause of the complete mortality of this group is unknown.

Apart from the direct relationship between temperature and the rate of development, two main conclusions can be made from these experiments. First, the eggs of *P. decipiens* can hatch in sea water at temperatures of 13° to 14° C.; they hatched into second-stage larvae. Secondly, the larvae were unable in 13 days to free themselves from the old cuticle of the first larval stage; from this it is inferred that entry into some intermediate host is probably necessary before the first cuticle can be dissolved.—D. M. Scott, *Atlantic Biological Station, St. Andrews, N. B. and Department of Zoology, University of Western Ontario, London, Ontario, Canada.*

A NYCTERIBIID FLY NEW TO ILLINOIS

On October 28, 1953, Dr. Philip W. Smith, Assistant Taxonomist, Illinois Natural History Survey, Urbana, and the author obtained 12 specimens of the Mississippi Bat, *Myotis austroriparius* from a limestone cave approximately one-half mile north of Rosiclare, Hardin County, Illinois. These bats, which represented a species new to Illinois, were equally divided and returned to the Natural History Survey and to the Illinois State Museum for further study.

Later inspection of these bats revealed that several fleas (*Myodopsylla insiginis*) and one fly were parasitizing the specimens; these ectoparasites were removed and preserved in 70% alcohol. The fly is a representative of the family Nycteribiidae, a family of small, spider-like, wingless flies all of which are parasites of bats. Through the courtesy of Dr. R. M. Stabler, Colorado College, Colorado Springs, the fly (a male) was identified as belonging to the genus *Basilia*. Since males lack good differential characters, the specimen was sent for species determination to Dr. L. R. Guimaraes, Departamento de Zoologia, Secretaria da Agricultura, Sao Paulo, Brazil, who now has a large amount of comparative material on hand and is completing a synopsis of New World Nycteribiidae.

Dr. Guimaraes identified this fly as *Basilia boardmani*, originally described by Rozeboom (1934, J. Parasit. **20**: 315–316) from specimens found on *Myotis austroriparius* in Florida. The occurrence of this fly in Illinois is of special interest since it is the first record of any representative of the Nycteribiidae in the state and extends the known range of the fly northward some 300 miles. Except for the Gulf-coast States, these parasitic flies have not been recorded east of the Mississippi River.

Although the cave near Rosiclare, as well as others throughout the state, was revisited on several later dates, and hibernating *M. austroriparius* and other species of bats were examined, no additional representatives of the Nycteribiidae were found.—Paul W. Parmalee, *Illinois State Museum, Springfield, Illinois.*

A MODIFIED TECHNIQUE FOR PREPARING WHOLE MOUNTS OF SIPHONAPTERA

The usual technique for preparing fleas for mounting on slides is both laborious and time-consuming. Most authorities recommend the following steps: bath in 10% KOH, either heating it for five to ten minutes or at room temperature for 24 hours or more; water, usually two or three changes; dehydration in several concentrations of alcohol, ending with absolute alcohol; clearing in oil of cedar, oil of wintergreen, or xylol; and mounting in Canada balsam. Workers at this college have developed a simplified technique which appears to give more consistently satisfactory results under all circumstances than the usual method.

The most critical part of the procedure, and the only step in which the specimen is likely to be damaged, is the clearing in KOH. To control this process more carefully, it is preferable to leave the KOH at room temperature. For light-colored species such as *Orchopeas leucopus*, 6 to 12 hours in KOH are sufficient. Dark forms such as *Cediopsylla simplex* may require 24 hours or more. In this technique, the fleas are not left in KOH until completely clear. When the critical structures are visible with reasonable clarity under a stereobinocular microscope, the fleas are removed from the KOH and placed in water for a few minutes. After a brief rinse, the specimens are transferred to Cellosolve. (Several chemicals are sold under the general name of Cellosolve. The one we have used is Ethyl Cellosolve, $C_2H_5OCH_2CH_2OH$.)

Use of Cellosolve eliminates all the succeeding steps prior to mounting. It dehydrates and hardens the specimen, at the same time completing the clearing. The specimen may be

left in Cellosolve indefinitely, until it is convenient for the worker to mount it. Twenty-four hours are usually sufficient to dehydrate and clear the fleas, but we have left specimens for two weeks without damage. Specimens may be transferred directly from Cellosolve to Canada balsam on the slide.

By reducing the time in KOH, and the number of times the specimen must be handled, this technique reduces the chance that the specimen will be damaged. The final clearing is left to the slow-acting Cellosolve, in which specimens will not over-clear if left for several days. By eliminating several steps, time and effort are saved, the specimens are more uniform, and hence more satisfactory for the taxonomist, than are specimens prepared by other methods. —ALLEN H. BENTON, *Department of Biology, New York State College for Teachers, Albany, New York.*

A CASE OF "MANGE" OF THE DOMESTIC RABBIT DUE TO *CHEYLETIELLA PARASITIVORAX* (MEGNIN)

In November, 1952 Mr. Truman Clark of this department called the attention of the writer to a domestic rabbit which appeared to have diseased fur. The rabbit was an adult (sex not determined) which had been caged in the laboratory for some time. Upon examination the animal was found to have an area of roughened fur in the scapular region, approximately 20 cm. in length and 7 cm. at its maximum width and extending from the base of the neck posteriorly along the mid-line of the back. The fur in this area was so loose that it could readily be pulled out in tufts.

Examination of the dishevelled fur with a dissecting microscope revealed a number of mites so the area was shaved, during which process a dense layer of material was found just above the skin. This layer was approximately 2 mm. thick and was composed of hair, detritus, mite exuviae, and mites in all stages of development. The skin over almost the entire area was inflamed and there was one smaller area of intense inflammation where exuded droplets of serum could be seen on the skin. No other areas of infestation could be seen on the animal and no other ectoparasites have been found in the infested material although a diligent search has been made. The shaved area was treated with benzyl benzoate and no subsequent cases have been seen in the rabbit colony.

Unfortunately the mite was not recognized at the time; it was subsequently identified as *C. parasitivorax* by Dr. G. W. Wharton of the University of Maryland. The pathogenicity of *Cheyletiella* has been questioned by many authors including Cooper (J. Parasit. **32:** 480–482, 1946) who, after a careful survey of the literature on this point, concluded that the mite's pathogenicity had not been established. The present case of "mange" appears to be identical with one described by Vail and Augustson (J. Parasit. **29:** 419–421, 1943) caused by *Eviugella americana* (syn. *C. parasitivorax*) in a domestic rabbit in California. The mite has been taken from rabbits (domestic and cottontail), hares, cats, and dogs. Olson and Roth (J. Parasit. **33:** 444–445, 1947) found large numbers of these mites on the scapular area of a cat from Baltimore, Maryland.

It appears to the author that the pathogenicity of *C. parasitivorax* can no longer be doubted since (1) the mite has been taken from a number of hosts on many different occasions, (2) heavy infestations are occasionally seen, sometimes, as in the present example, in the apparent absence of other ectoparasites, (3) the mites have been found associated with definite lesions in at least two cases (that of Vail and Augustson and the present one), and (4) the mites occur in the scapular region on such diverse animals as rabbits (in the study of Vail and Augustson and the present one) and cats.

This is Paper No. 3292 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota.—A. RALPH BARR, *Department of Entomology and Economic Zoology, University of Minnesota.*

THE OCCURRENCE OF *NEOECHINORHYNCHUS CRISTATUS* LYNCH, 1936, (ACANTHOCEPHALA) IN EASTERN NORTH AMERICA

Since its original description by Lynch (1936, Tr. Am. Micr. Soc. **55:** 21–43) *Neoechinorhynchus cristatus* has been reported from several species of suckers in western North America. Locality references include Washington state (Lynch, 1936); California (Haderlie, 1953, Univ. of Calif. Publ. in Zool. **57:** 303–440); and British Columbia (Bangham and Adams 1954, J. Res. Bd. Canada **11:** 673–708). The latter authors, as well as Van Cleave (1949, J. Parasit. **35:** 500–512) and Van Cleave and Haderlie (1950, J. Parasit. **36:** 169–173), have emphasized the unique speciation of the NEOECHINORHYNCHIDAE in western North America. Until now *N. cristatus, N. venustus,* and *Octospinifer torosus* have been thought to represent a distinctive acanthocephalan fauna of the CATOSTOMIDAE west of the Rockies.

In the fall of 1953 a class survey was made of the parasites of the fishes in the Bellamy River in Madbury, New Hampshire. It was found that nearly all the specimens of the white sucker, *Catostomus commersonii*, were infected with a species of *Neoechinorhynchus* which closely resembled *N. cristatus* in the presence of a prominent keel along the dorsal surface. Since that time numerous collections of these suckers have yielded many specimens of this acanthocephalan from several widely separated localities in southeastern New Hampshire. To date the parasite has been found in all the major streams of the coastal watershed as well as in the one pond that was checked in the Merrimac watershed. The parasite was associated with *Pomphorhynchus bulbocolli* and/or *Octospinifer macilentis*. The incidence was higher in young fish, and, although no specimens of this species have been collected in suckers examined in the spring of the year, not enough data has been accumulated to determine if this represents a true seasonal distribution.

In the light of a considerable gap in the geographic distribution of this parasite and *N. cristatus*, it was thought advisable that, before any definite species assignment, a detailed study should be made of the specimens collected. Through the courtesy of Dr. J. F. Lynch a series of his Washington specimens were available and could be measured for comparison with the New Hampshire material. These measurements were as indicated in Table I.

TABLE I.—*Measurements of three series of* Neoechinorhynchus.

		N. cristatus Lynch, 1936	*N. cristatus* Washington sample	*N. sp.* New Hampshire material
Hook length in mm.	Apical row	0.045–0.059	0.038–0.059	0.035–0.062
	Middle row	0.026–0.038	0.024–0.035	0.018–0.028
	Basal row	0.023–0.032	0.020–0.027	0.014–0.024
Length of lemnisci in % of total body length	Short lemniscus, male	21–38%	19–39%	10–21%
	female	12–22%	10–25%	8–13%
	Long lemniscus, male	31–64%	31–48%	19–36%
	female	23–35%	21–40%	10–21%
Length of male genitalia in % of total body length		———	52–73%	45–63%
Dimensions of embryos in body cavity of stained females		38 by 16 microns (average)	37–39 by 14–18 microns	36–39 by 14–16 microns

From the data it can be seen that the eastern series of specimens exhibits: 1. slightly smaller hook size; 2. smaller lemnisci; and 3. smaller body space occupied by the male genitalia. However, in all these features there is a pronounced overlap between the eastern and western materials. A similar overlap was reported by Lynch (1936) in his careful study of *N. crassus* from Washington and material supplied by Van Cleave from the midwest. It is, therefore, concluded that, in spite of the apparent discontinuity in distribution the New Hampshire material conforms to the description of *Neoechinorhynchus cristatus* Lynch, 1936. Until further information is available on the distribution of the ACANTHOCEPHALA in catostomid fishes it does not seem advisable to attempt to explain this unique distribution.—WILBUR L. BULLOCK, *Zoology Department, University of New Hampshire, Durham.*

AN APPARENT HUMAN CASE OF MYIASIS BY THE HORN FLY, *SIPHONA IRRITANS*

A muscoid larva, submitted to this laboratory by Brooke Army Hospital, Fort Sam Houston, Texas, was tentatively identified as a third instar stage of the horn fly, *Siphona irritans* Linn. The specimen had been expressed from an abscess in the groin region of a patient recently admitted to the hospital for treatment. The identification was confirmed by Drs. H. J. Reinhard and Donald R. King of the Department of Entomology at Texas Agricultural and Mechanical College to whom we express our appreciation.

A summary of the case history of the patient relates the following: "This 54-year-old Negro male was admitted to Brooke Army Hospital following evacuation by ambulance from Cuero, Texas. The patient speaks most unintelligibly and history is unobtainable at this time. The main complaints include pain in the left lower quadrant of the abdomen on coughing, soreness around anal region, soreness around the scrotum, bad cough. There is a 3 mm. white larva found in the right groin area."

The patient was reported to be extremely filthy, and it is assumed that he may have been living in either a barn or stable. His body was reportedly caked with dirt.

There is no previous record of this fly having produced myiasis in man.—STUART E. KNAPP, GEORGE M. PADILLA, AND F. M. PHILIPS, *Fourth Army Area Medical Laboratory, Brooke Army Medical Center, Fort Sam Houston, Texas.*

PARASITES OF THE COYOTE *CANIS LATRANS* SAY, IN KANSAS

Advantage was taken of an extensive study at Kansas State College of the embryology and food habits of the coyote, *Canis latrans* Say, to examine this host for parasites. The study covered a period of 7 years (1948–1954) during which 1,142 coyotes were examined. Most of the animals were brought in during the winter months and were obtained largely as the result of coyote drives, mostly within a 75 mile radius of Manhattan though various numbers of coyotes were sent in from all areas of the state.

The most numerous species of helminth was *Taenia pisiformis* which was recovered from 92.8% of the coyotes. *Dipylidium caninum* was found in three animals, a total of 6 specimens being collected. The stomach worm, *Physaloptera*, was the most numerous nematode, being found in 60.4% of the animals. The species were determined only in 1948. *P. rara* was recovered from 255 animals and *P. praeputialis* from 3. *Toxascaris leonina* occurred in 19.3% of the coyotes and 19.8% contained *Ancylostoma caninum*. The heartworm, *Dirofilaria immitis*, has not previously been reported from Kansas but was found in 8 of 954 animals examined. The most heavily infected heart contained 31 worms. *Trichuris vulpis* was recovered from the ceca of 7 of 63 animals carefully examined for this species.

The following species of fleas were collected from 53 coyotes: *Pulex irritans, Ctenocephalides canis, Cediopsylla simplex* and *Hoplopsyllus affinis*.—Donald J. Ameel, *Zoology Department, Kansas State College, Manhattan, Kansas.*

ESTERASE ACTIVITY IN *ENDAMOEBA HISTOLYTICA*

Evidence has been obtained for the presence of an esterase in *Endamoeba histolytica*. The hydrolysis of methyl n-butyrate by the test substance, with acid production, was the system used to detect the presence of this enzyme.

The amebae studied were from cultures of the DKB strain of *E. histolytica* grown monobacterially with *Escherichia coli*, in Balamuth's medium. The amebae were sedimented, thrice washed in normal saline, and were lysed by alternately freezing and thawing. Flora controls (*Escherichia coli*) were cultured and prepared under conditions identical to those which were used with the amebae.

Methyl n-butyrate, 0.1% in 0.4N glycine buffer, *p*H 8.7, was the substrate used. Hydrolysis was tested by mixing 0.7 ml of test substance with 0.7 ml of substrate; and the exposure time was 24 hrs. at 37° C. The reaction was stopped by addition of 5 ml of a phenol indicator prepared by mixing 1.5 ml of 0.4% aqueous bromthymol blue with 10 ml of 2% phenol. Loss in alkalinity, due to acid production, was determined by titration to *p*H 6.5 with the aid of a *p*H meter, using 0.05 N HCl.

Plate counts to determine numbers of viable bacteria present were made on the ameba lysate and the flora control at the start of each assay. Flora controls were found always to contain bacteria in numbers equivalent to, or greater than, those present in the ameba lysates. In addition to the flora control, other controls used were: 1) a reagent blank, and 2) ameba lysate incubated with glycine buffer without added methyl n-butyrate ("starch control"). The latter control was required due to the unavoidable presence in the ameba lysate of residual rice starch, which it was believed might contribute to a *p*H shift.

The results of 8 experiments are tabulated in Table I. It is to be noted that all values in

Table I. *Milliliters of 0.05N HCl required to adjust to pH 6.5*

Experiment no.	Ameba lysate	Flora control	Starch control	Reagent blank
1	0.25	0.50	0.55	0.45
2	0.25	0.495	0.45	0.455
3	0.30	0.40	0.45	0.50
4	0.145	0.40	0.55	0.545
5	0.00	0.20	0.25	0.35
6	0.00	0.30	0.20	0.35
7	0.20	0.35	0.35	0.45
8	0.15	0.25	0.40	0.45
Averages	0.162	0.362	0.40	0.444

Experiments 5 and 6 are lower than those obtained in the other assays, including all controls. The reason for this variation is unexplained. In all experiments, however, including those with low values, acid was produced from the substrate by the ameba lysate in significantly greater amounts than in the various controls.

This work was carried out under the sponsorship of the Commission on Enteric Infections, Armed Forces Epidemiology Board, and was supported by the Office of the Surgeon General, Department of the Army.—F. A. Hallman, J. N. DeLamater, and J. B. Michaelson, *Depart-*

ments of Microbiology, Medicine, and Biochemistry, University of Southern California School of Medicine, Los Angeles 7, and the Institute of Medical Research, Huntington Memorial Hospital, Pasadena 2, California.

CORYNOSOMA HADWENI VAN CLEAVE A PROBABLE SYNONYM OF *C. WEGENERI* HEINZE (ACANTHOCEPHALA)

Van Cleave (1953a, J. Parasit. **39:** 1–13; 1953b, Illinois Biol. Monogr. **23:** 1–179) described as a new acanthocephalan species, *Corynosoma hadweni*, from seals caught mainly in the vicinity of Alaska. In his monograph on the ACANTHOCEPHALA of North American mammals, Van Cleave (1953b) listed the following definitive hosts of *C. hadweni*: *Halichoerus grypus* from Unalaska, Alaska; *Phoca hispida* from Point Barrow and Kotzebue, Alaska, and from Baffin Island, Eastern Canadian Arctic; *Erignathus barbatus* from Point Barrow, Alaska; *Phoca* sp. and unidentified seals from Kotzebue, Unalakeet, and St. Lawrence Island, Alaska. He also identified as *C. hadweni*, larvae (cystacanths) from visceral cysts in a fish (*Osmerus mordax*) collected from the State of Maine, thus indicating that the species subsequently would be found in seals of the western North Atlantic.

TABLE 1. *Comparison of* C. hadweni *and* C. wegeneri.

All measurements in millimeters. Measurements and numbers of hooks deduced from illustrations are placed in parentheses.

	C. wegeneri	*C. hadweni*
Total body length male and female	4.5	6–8.25
Max. diam. fore-trunk male	1.02	1.43
female	1.02	1.43–2.50
Diam. hind-trunk	0.25–0.35	1/5–2/5 fore-trunk
Trunk spines	Ventrally extend posteriorly about ⅛ hind-trunk	Ventrally usually extend not much beyond swollen fore-trunk
	(0.024–0.035 by 0.007–0.008)	0.026–0.046 by 0.008–0.014
Genital spines, male	Present—no measurements	0.024–0.035 by 0.010
Proboscis— Length	0.84	0.84–0.93
Max. diam.	0.275	0.30–0.37
Diam. anteriorly	0.20	0.23
Proboscis hooks— No. long rows	15 or 16	16
No. hooks in each row	10 or 11	10 or 11 (9–11)
No. simple hooks in each row	4 (4 or 5)	5 or 6 (3–5)
No. hooks with recurved roots in each row	6 (6 or 7)	(5–7)
Size of proboscis hooks— Near distal end	(0.045–0.070 by 0.006–0.014)	0.079–0.104 by 0.004–0.016
On swollen region	(0.074–0.090 by 0.024–0.035)	0.105–0.150 by 0.026–0.041
Basal hooks	(0.033–0.051 by 0.004–0.010)	0.046–0.061 by 0.005–0.011
Embryos	0.108 by 0.030	0.093–0.115 by 0.020–0.035

Kruidenier (1954, J. Parasit. **40:** 363–364) pointed out that *H. grypus* does not occur in Alaskan waters and hence Van Cleave's records of ACANTHOCEPHALA in that host in Alaska are incorrect. He further suggested that the seals referred to as *H. grypus* were almost certainly *Phoca vitulina richardi*.

In his papers, Van Cleave discussed the species of *Corynosoma* found in PINNIPEDIA of the Northern Hemisphere, but apparently overlooked *Corynosoma wegeneri* Heinze (Heinze, K. 1934, Wissensch. Ergebn. Deutschen Grönland—Expedition Alfred Wegener, 1929 und 1930–31 **6:** 185–189, Leipzig) from *Phoca hispida* of Greenland. In comparing the descriptions of *C. hadweni* and *C. wegeneri* there is little, if anything, that distinguishes the two species. The description of *C. wegeneri* lacks certain details, particularly measurements of proboscis hooks and body spines. Nevertheless it still appears that *C. hadweni* and *C. wegeneri* are conspecific, with *C. hadweni* falling as a synonym of *C. wegeneri*.

In the accompanying table a comparison is made between *C. wegeneri* and *C. hadweni*. Measurements of proboscis hooks and body spines of Heinze's specimens had to be calculated from his illustrations. These measurements are, generally, smaller than the corresponding ones given by Van Cleave for *C. hadweni*. However, measurements based on calculations from

illustrations, particularly of small structures, cannot be relied upon for accuracy. Furthermore, Heinze's illustration of the proboscis, according to the accompanying scale, indicates that this structure is only 0.7 mm. in length by about 0.25 mm. in maximum width. In the text Heinze gives the length and maximum width of the proboscis as 0.84 mm. and 0.275 mm. respectively. On the presumption that the illustration is of the same specimen to which measurements in the text apply, it appears that the figure of the proboscis and hooks was drawn to a smaller scale than indicated, and consequently the hook sizes calculated from the illustration are considerably less than their actual sizes. Further discrepancies are evident between the text and illustrations of the number of proboscis hooks in both *C. hadweni* and *C. wegeneri*. These are indicated in the table.—Leo Margolis—*Pacific Biological Station, Nanaimo, B. C., Canada.*

NOTES ON THE LIFE CYCLE OF *FIBRICOLA CRATERA* (TREMATODA: STRIGEIDA)

The life cycle of *Fibricola cratera* (Barker and Noll, 1915) Dubois, 1932 was reported in 1946 (Cuckler, A. C., J. Parasitol. **26** (6-Suppl.): 32–33). The writer repeated the life cycle at the University of Iowa in 1948–49 and wishes to add a few observations. Apparently the life cycle of *Fibricola texensis* Chandler, 1942 (Chandler, A. C. 1942, Tr. Am. Micr. Soc. **61**: 156–167) is very nearly identical to that of *F. cratera*. The genus has been reviewed by C. P. Read (Tr. Am. Micr. Soc. **67**: 165–168, 1948).

Ova were obtained from the feces of three raccoons, *Procyon lotor*, infected with *F. cratera*. Nine ova ranged from 99 to 125 microns by 70 to 76 microns, mean 108 by 73 microns. Four lots of ova were incubated at room temperature (21 to 24° C) and miracidia first appeared respectively in 9, 10, 14, and 14 days. It was possible to store unembryonated ova at 4 to 6°C safely for 49 days and to store embryonated ova safely for 44 days. Hatching occurred in the dark as well as in the light.

The miracidium was very similar to that of *F. texensis*. A maximum longevity of 7 hours was observed. Miracidia of *F. cratera* were killed in 1: 512,000 copper sulfate in 1 hour or less.

Fifteen laboratory reared *Physa gyrina* were exposed to miracidia; all but three of the snails died and only one of the three became infected. Cercariae were first seen at 30 days. Cercariae were also obtained from naturally infected *Physa gyrina* and *P. sayii*, from Lake Macbride, Iowa City, Iowa, and Carrol Lake, Woodruff, Wisconsin, respectively. Without refrigeration, infected snails could be kept alive for only 7 to 14 days, but by refrigerating during the time when cercariae were not needed, they could be kept alive and producing cercariae as long as 45 days.

The maximum longevity of the cercariae was 52 hours and they were infective for tadpoles at least 21 hours (probably longer) after emerging. Cercariae emerged in the dark as well as in the light. Cercariae would not penetrate frog embryos prior to hatching, but readily penetrated tadpoles one day old and older. Tadpoles of *Rana pipiens*, *Pseudacris nigrita tricerata*, and *R. clamitans* were infected; those of the latter species were approximately 1 year old. Adult *R. pipiens*, *Acris crepitans*, and *Bufo a. americanus* did not become infected after exposure to the cercariae. It was not possible to infect any of six species of fish with the cercariae. The metacercariae were free in the body cavity of the tadpoles from 12.5 hours or less until shortly after metamorphosis of the tadpoles, when the metacercariae migrated into the hind legs and became encapsulated. This was reported by Cuckler for *F. cratera*, but Chandler found that the metacercariae of *F. texensis* did not leave the body cavity. It was not possible to infect fish by feeding them metacercariae or by injecting metacercariae into the body cavity.

Since metacercariae could be recovered easily from frog legs by the pepsin digest method (3,550 were recovered from one *Rana pipiens*), the time at which the ability to withstand pepsin-HCl solution became evident was determined. Up to and including 8 days of age the metacercariae perished in less than 1 hour in the pepsin solution at 37°C. At 10 days a very small percentage survived 8 hours, but many fully mature metacercariae from frog legs survived as long as 24 hours.

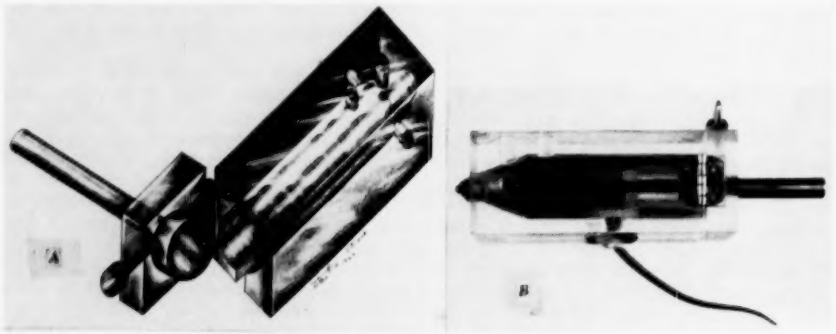
It was possible to infect laboratory rats with 35 day old metacercariae, but not with those 15, 18, or 22 days old. One young Syrian hamster, *Cricetus auratus*, was experimentally infected and yielded two adult worms when autopsied 60 days later. One domestic pigeon was fed 60 infective-age metacercariae but did not become infected.

I wish to thank Dr. L. O. Nolf, University of Iowa, Iowa City, for his interest, encouragement, and advice during the course of this work.—Glenn L. Hoffman, *University of North Dakota.*

DESCRIPTION OF A PLASTIC MOUSE RESTRAINING CASE

Exposure of mice by tail to known numbers of cercariae of *Schistosoma mansoni* by the method of Olivier and Stirewalt (1952, J. Parasit. **38**: 19–23) is facilitated by replacing the restraining boards with clear plastic restraining cases (Fig. 1 A and B). These cases were bored out of plastic blocks measuring 9 by 3.8 by 3.8 cm. A ventral slit accommodates the tail. One end of the tube, that with the tapered bore, holds the anterior end of the mouse and provides for breathing through a terminal opening of small diameter. The opposite terminal opening, through which the mouse enters the case, is closed by a hinged door through which a piston slides to hold the mouse in the graduated end of the tube and to accommodate the length of the tube to the size of the mouse. The piston, pushed against the mouse and held in place by a wing screw, also holds the hinged door shut. Six small holes bored through the plastic provide ventilation.

After encasement, the mouse is restrained further by two strips of adhesive around the tail as it emerges from the case. One long edge of each strip is pressed against the mouse's tail and the other against the wall of the case in such a way that withdrawal of the tail into the case is prevented.



Plastic mouse restrainer. A, open; B, with mouse in place.

Case dimensions are as follows: length, including hinged door, 10.5 cm.; width and height, 3.8 cm.; door thickness, 1.2 cm.; diameter of tube at open end, 2.5 cm. The same bore extends for a depth of 7 cm., at which point it begins to taper to a final diameter of 1 cm. at the terminal opening.

The usefulness of the restraining case is not limited to exposures of mouse tails to schistosomes, but extends to any procedure in which the mouse tail is to be manipulated.—M. A. Stirewalt and J. F. Bronson, *Naval Medical Research Institute, Bethesda, Maryland.*

SURVIVAL OF FROG LUNG FLUKES, *HAEMATOLOECHUS* SP., IN CERTAIN ARTIFICIAL MEDIA

Sixty-one adult *Haematoloechus* sp. from 20 *Rana pipiens* were transferred to artificial media (58 cultures) and survival times studied. Bacteria-free cultures were obtained relatively easily, but none of the media tried was adequate to maintain the flukes.

Bacto Nutrient Agar, made according to formula, and amphibian Ringer's solution (0.65% NaCl, 0.025% KCl and 0.03% $CaCl_2$) were the basic media. Supplements of 0.5% dextrose, 0.25% Bacto yeast extract, and 0.25% hemoglobin powder, singly or in combination, were added to the agar. In some tests flukes were kept on the surface of agar plates, dampening them with Ringer's as necessary. Usually the agar was tubed and covered with an overlay of Ringer's. Fresh frog tissue—liver, heart or kidney—was added to some tubes. A few tests were made with bacteriological dextrose broth (Difco).

Aseptic techniques were used during removal of trematodes from the lungs. Some flukes were put into culture media directly from the Ringer's in which the lungs were opened; others were rinsed through 1 to 3 washes of Ringer's in petri dishes. A bacteriological needle with tip bent at right angles was used to move the worms, sterilizing it before each transfer.

Incubation was at room temperature as *Haematoloechus* dies at 37°C. Six cultures put in an incubator at this temperature were dead when examined less than 4 hours later. Two were on agar plates, two in dextrose broth, and the others in Ringer's over agar.

Bacteria-free trematodes (meaning free of aërobic organisms capable of growing in the media used) were obtained more easily than had been anticipated. Some cultures started by transferring worms directly from the Ringer's in which the lungs were opened remained sterile. Of the 58 original isolations, 31 remained sterile, 3 additional were agar plates with a single contaminating colony not close to the fluke, and 1 culture in plain Ringer's was called doubtfully contaminated. In general, contaminations were most frequent in cultures subjected to numerous manipulations.

Four worms were kept separately in tubes containing about 5 ml. of Ringer's. Two, both in tubes with slight bacterial growth, lived 7 days, one 9 and the other 16 days.

One fluke lived 2 days in sterile dextrose broth. Trematodes in 5 contaminated cultures were inactive before bacterial growth became heavy.

All tubes containing fresh frog tissue developed heavy bacterial growth and the trematodes died in less than 2 days.

Ten out of 41 individuals in Ringer's solution over agar lived 12 days or longer. Three survived 12 days, and one each 13, 16, 17, 18 and 21 days without change of medium. In 2 other tubes the Ringer's was replaced with fresh solution on the 19th day. One of these flukes lived 27 days, the other 28.

Agar plate cultures were lost more rapidly because of excessive drying or contamination, but worms were less active and less emaciated than those in fluid cultures.

No definite feeding movements were seen except faint contractions of the oral sucker musculature of one specimen in a liver culture. The ceca of this individual contained a slight amount of material which appeared to have been ingested from the medium. In one petri dish culture, the surface of the agar was marked with rows of circular prints made by the oral sucker. These looked like the result of attempts to anchor the body for locomotion on the drying surface, rather than ingestion of substrate.

In general, pieces of trematode lacking only the anterior end survived about as well as whole worms and some appeared less emaciated than unbroken individuals treated in the same manner, possibly because pieces were less active. The longest survival time for a piece was 17 days. No regeneration of any part of the anterior end occurred in cut specimens.

Microscopic examination of eggs showed the contents reduced to granular debris. Apparently no new eggs were formed after the flukes were removed from the frogs. Certainly deterioration of the gonads is already evident within the first two or three days as the parenchyma disappears.—HELEN M. CHURCHILL, *Department of Biology, Hollins College, Virginia.*

A SIMPLIFIED METHOD FOR THE CONCENTRATION OF CERCARIAE

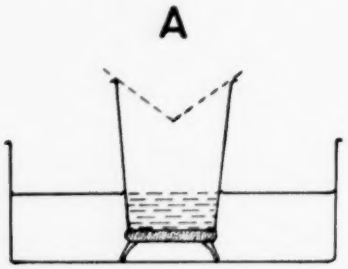
The use of cercarial antigens for diagnostic purposes in schistosomiasis is greatly handicapped by the lack of an efficient method for obtaining heavy cercarial suspensions relatively free from contamination (snail feces, unicellular algae, free-living Protozoa, small crustaceans, etc.)

The centrifugation of active cercariae suspensions (Oliver-Gonzáles and Pratt, 1944, Puerto Rico J. Pub. Health and Trop Med. **20:** 242–256) and the narcotizing of the cercariae with diluted ethyl alcohol (Bozicevich and Hoyem, 1947, Bull. [189] Nat. Inst. Health, pp. 199–212) or sulfuric ether (Martins, 1949. Thesis. Fac. Med. Univ. Minas Gerais, Brazil. 265 pp.) for causing them to sink proved to be unsuitable; the concentration obtained is poor and the unwanted contaminants are also carried down. The method of "accelerated vertical migration" proposed by Standen (1950, Tr. Roy. Soc. Trop. Med. and Hyg. **43:** 527–530; 1952, J. Helminthol. **26:** 25–42) provided a more reliable process of cercarial concentration, but an important part of the material may be lost in the successive dilutions and the separation of the contaminants is not always satisfactory.

The following method is being used with very good results, in the Laboratory of the Serviço Especial de Saúde Pública, Minas Gerais, Brazil, to concentrate cercariae of *Schistosoma mansoni* discharged by infected *Australorbis glabratus.*

The cercariae-producing snails are carefully washed in tap water and their shells thoroughly cleaned with a soft tooth-brush. The purpose of this operation is to remove organic and inorganic débris that is usually on the snail's shell and constitutes an important source of contamination during the concentrating process. It is recommended to use thin rubber gloves for handling the snails and to brush the shells in a crystallizing dish filled with water to avoid the spreading of infected material. The cleaned snails are rinsed in tap water and dispersed into beakers of 100 ml. capacity. Five or six snails can be put together in a single beaker with 50 ml. of filtered spring water. The snails are exposed to indirect sunlight for two hours or more until a dense cercarial suspension is obtained.

When a limited volume of heavy cercarial suspension is desired, the cercarial suspension contained in the beakers is carefully transferred to crucibles fitted with permanent porous plates



(sintered glass crucibles, Fig. A). Crucibles with discs of medium porosity (number 3; pore diameter ranging from 20 to 30 micra) and with 20 to 30 ml. capacity are recommended. The crucible is placed in a crystallizing dish so that the water will flow spontaneously and the cercarial suspension is then concentrated. The degree of the concentration can be easily controlled by changing the water level in the crystallizing dish. For obtaining relatively pure cercarial suspensions it is advisable to remove the snails from the beakers and to filter the cercarial suspensions through a wire gauze (40 mesh to the inch) while transferring them to the crucible. A Büchner funnel with fused-in fritted disc of medium porosity adapted to a suction flask proved to be very practical in obtaining large amounts of heavy cercarial suspensions. When using Büchner funnels care must be taken to obtain a moderate filtration rate in order to prevent cercarial destruction.—J. PELLEGRINO, *Departamento de Parasitologia, Faculdade de Ciências Médicas, Belo Horizonte, Brasil* AND D. G. MACEDO, *Serviço Especial de Saúde Pública, Belo Horizonte, Brasil.*

INCIDENCE OF *ENTEROBIUS VERMICULARIS* IN 535 PUERTO RICAN CHILDREN

A pinworm survey on 535 Puerto Rican children was carried out during the period from July 13 though August 5, 1954. This is the first pinworm study on a large group of Puerto Rican children from the general population. Jeffery (1950, J. Parasit. **36**: 485–488) reported the incidence of pinworm in 84 Puerto Rican children from the general population. One hundred-two children from institutions in Puerto Rico were examined for enterobiasis by Brady (1941, Proc. Helminth. Soc. Wash. **8**: 10).

This pinworm survey was done on children at the pediatrics outpatient clinic of the Municipal Hospital in Santurce, Puerto Rico. All of the children, ages one through 14 years, came from families of low incomes in or near the various slum areas or recently moved from slum areas of Santurce, Puerto Rico.

Petersen and Fahey's (1945, J. Lab. & Clin. Med. **30**: 259–261) modification of the Scotch Cellulose Tape method described by Graham (1941, Am. J. Trop. Med. **21**: 159–161) was employed in this survey. One Scotch Cellulose Tape swab was obtained from each child between the hours of 8:30 A.M. and 10:00 A.M. The usual instructions as to bathing and defecation were not given because the children were chosen at random from the outpatient clinic to which they came for reasons other than the survey.

In general it has been observed that the incidence of *Enterobius vermicularis* in tropical countries in children from the general population is low. Brady (1941; Proc. Helminth. Soc. Wash. **8**: 10) stated several possible reasons for the low pinworm incidence in the tropics. Enterobiasis in Filipino school children was 75.2 percent, (Chanco and Sariano, 1939 Acta. Med. Phil. **1**: 81–89). Mazzotti (1945, Rev. Inst. Salub. y Enferm. Trop. Mexico **6**: 37–40) observed the incidence of enterobiasis in children in tropical Mexico to be 13.48 percent. However, its occurrence in nontropical Mexico was reported as 48.0 percent, (Mazzotti and Quintanar, 1943, Rev. Inst. Salub. y Enferm. Trop. Mexico **4**: 173–178). Stoll, Chenoweth and Peck (1947, Puerto Rico J. Pub. Health & Trop. Med. **22**: 235–243) reported one percent infection in the natives of Guam. Pinworm swabs on 102 children from institutions in Puerto Rico showed a 21.0 percent incidence, (Brady, 1941, Proc. Helm. Soc. Wash. **8**: 10). An 11.9 percent occurrence of pinworm was reported by Jeffery (1950; J. Parasit. **36**: 485–488) in Puerto Rican children. The results of this study on 535 Puerto Rican children showed a 6.0 percent minimum incidence of pinworn infection.

Eggs of *Ascaris lumbricoides* and *Trichuris trichiura* were also encountered on the Scotch Tape swabs. A 16.8 percent incidence of *Ascaris* and a 15.9 percent occurrence of *Trichuris* were observed in the 535 children examined. The incidence of multiple infections was 7.2

percent. Jeffery (1950; J. Parasit. **36**: 485–488) observed a 9.15 percent *Ascaris* infection and a 16.7 percent *Trichuris* infection in Puerto Rican children with Scotch Cellulose Tape method.

The incidence of *Ascaris*, *Trichuris* and *Enterobius* was slightly higher in females than in males. In 290 females examined the incidence was 48.3 percent, whereas in 245 males the incidence was 42.9 percent. Brady (1941, Proc. Helminth. Soc. Wash. **8**: 10) reported a 30.0 percent occurrence of pinworm infection in institutionalized females as compared to a 12.0 percent infection in institutionalized males. The occurrence of multiple infections was also slightly higher in females than in males (Table I).

TABLE I.—*Incidence of parasitism in 535 Puerto Rican children examined by Scotch Tape swab*

Age group	Total number of children examined	Total number of males examined	Infection in males				Total number of females examined	Infection in females				Total number of infections			
			A*	T*	E*	M*		A*	T*	E*	M*	A*	T*	E*	M*
1– 2	187	72	11	7	2	3	115	20	19	9	12	31	26	11	15
3– 4	151	69	12	11	5	5	82	16	15	8	3	28	26	13	8
5– 6	85	46	9	11	3	6	39	4	9	2	3	13	20	5	9
7– 8	60	28	7	5	1	2	32	3	2	1	2	10	7	2	4
9–10	33	17	2	2	1	1	16	4	4	0	2	6	6	1	3
11–12	15	10	0	0	0	0	5	2	0	0	0	2	0	0	0
13–14	4	3	0	0	0	0	1	0	0	0	0	0	0	0	0
Totals	535	245	41	36	12	17	290	49	49	20	22	90	85	32	39
Percent infection			16.7	14.7	4.9	6.9		16.9	16.9	6.9	7.6	16.8	15.9	6.0	7.2

* A—*Ascaris*, T—*Trichuris*, E—*Enterobius*, M—Multiple infections.

Mazzotti (1945, Rev. Inst. Salub. y Enferm. Trop. Mexico **6**: 37–40) observed the highest incidence of pinworm in children between the ages of 6 and 7. Children in the 3 through 4 age group showed the highest pinworm incidence and the highest total parasite infection in this survey (Table I).—THOMAS D. MALEWITZ, *Department of Anatomy, Michigan State College, East Lansing, Michigan*, and MICHAEL G. LYSENKO, *Departments of Microbiology and of Zoology, University of Wisconsin, Madison, Wisconsin.*

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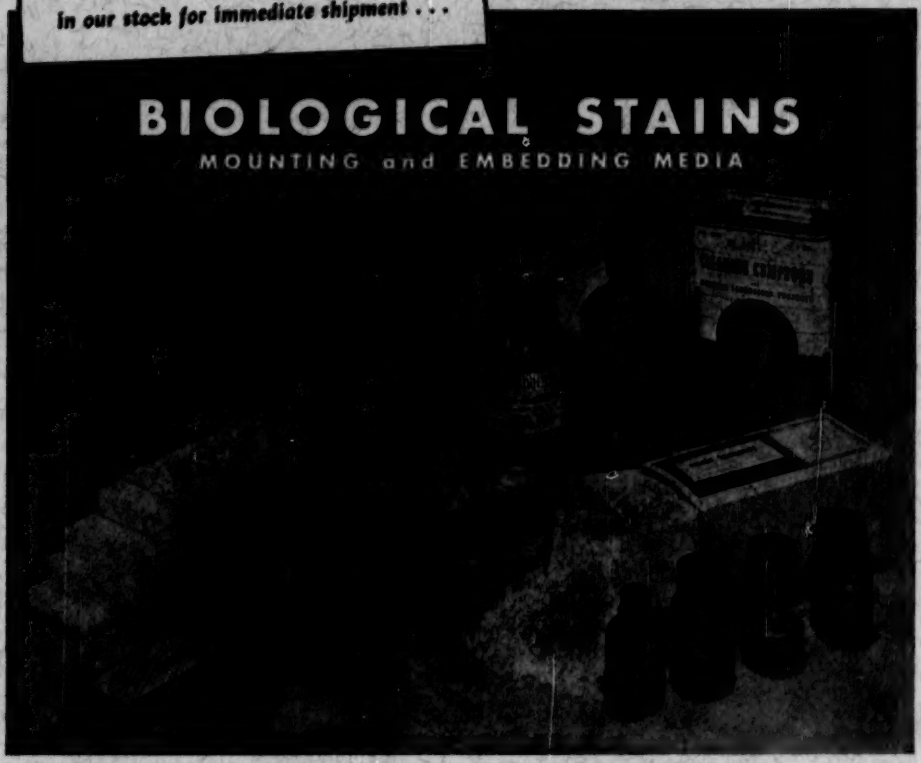

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